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THE INFLUENCE OF THE DECOMPOSITION OF ORGANIC MATTER ON THE OXIDATION-REDUCTION POTENTIAL OF SOILS[1]

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The study of potentials developed in soils, begun by Gillespie in 1920 (6), has been continued sporadically through the following years. Considerable progress has been made in this connection, as brought out by the work of Remezow (13), Willis (14), Heintze (9, 10), Brown (3), Bradfield, Batjer, and Oskamp (2), and Peech and Batjer (11). The E_h-pH relationship, the relation of potential to the formation of ferrous compounds, and the relation of potential to fertility in orchard soils have been carefully studied. From a practical soil point of view, the last may be particularly significant. As far as the authors are aware, however, no effort has been made to study the development of oxidation-reduction potential in relation to the decomposition of various types of organic matter in standing soil. In view of the theoretical and possible practical importance of this problem in soil investigation the present experimental work has been undertaken in an effort to throw some light on the effect of such decomposition and attendant microbial activity on the reducing intensity of the soil.

The observations recorded here are corrected to pH 7.0. The intimate relation between pH and E_h makes some degree of correction esssential when pH varies as widely as in the present experiments. Willis (14), making measurements on soils suspended in water, found a pH-E_h slope of 0.060 volt per unit change in pH. This is in good agreement with the theoretical value 0.059 for the quinone-hydroquinone system and certain other systems in which an exchange of two electrons is involved. Peech and Batjer (11), however, found a somewhat steeper slope when measurements were made on soil suspended in 0.1 *N* H_2SO_4 and used a correction factor of 0.080 volts per unit change in pH. They discuss the degrees of slope that accompany various oxidation-reduction systems in which varying numbers of electrons are concerned as well as the conditions of their own experiments, such as the evolution of CO_2. In the present experiments the smaller factor 0.060 is used in

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order to minimize the differences in potential level attained in the presence of organic substances of a protein or carbohydrate nature. At present any correction factor can be no more than a guess. This uncertainty is particularly true in the experiments reported here in which oxidation of substrates differing widely in their nature is taking place—very possibly different systems with different E_h-pH relations are involved (1).

The practice of suspending soil in water or dilute acid solutions prior to measuring oxidation-reduction potential is open to some criticism. It is quite possible that dissolved oxygen in such liquids will affect relatively poorly poised systems to a considerable degree. In the experiments under consideration here wide and unpredictable fluctuations have been observed, not only in comparing values obtained from direct measurements on soil with those obtained on water suspensions of the same soil (prepared for pH determinations), but also among duplicate samples of soil subjected to such treatment. We have, therefore, endeavored to make potential measurements on soil samples disturbed as little as possible. In this way, fairly good agreement between duplicate pots has been obtained. It may be added in this connection that sand media give much closer agreement between duplicate electrodes and duplicate pots than do soil mixtures and are, therefore, preferable to the latter.

EXPERIMENTAL

The electrodes were bright platinum wire, 1.5 inches long. These were inserted directly into the soil sample. An attempt was made to use bright platinum plates made rigid by means of a sealed on glass support, but these were unsatisfactory. Duplicate electrodes were used throughout. The salt bridge, which was also inserted into the soil sample, was a saturated solution of KCl so arranged that the entire bridge could be flushed out with fresh salt solution after each measurement. As will be pointed out later, it is of the greatest importance that the bridge be thoroughly cleaned between samples, an operation that is not possible with agar bridges. The remainder of the apparatus was of the usual type [that used here has been described (4)].

The soil used in these experiments was a sassafras sandy loam. Preliminary tests indicated that potential measurements could be made on such a soil when it contained 20 per cent or more moisture. The optimum moisture content for the activities of aerobic organisms, namely, 20 to 24 per cent, was, therefore, used throughout the experiments. The added material, casein, starch, etc., was mixed with the dry soil, and the mixture was placed in pots. Distilled water was added to bring the moisture content to the desired level. Two to three hours was allowed to elapse between the addition of water and the first removal of samples. Presumably during this time the moisture became more or less uniformly distributed, and it was assumed that no activity capable of bringing about changes in oxidation-reduction potential had taken place, the time of removal of the first sample being recorded as 0 hours. Every experiment was run with duplicate pots each of which contained

1000 to 1500 gm. of soil, on a dry basis. The pots were covered with glass plates and kept at a constant temperature of 28°C.

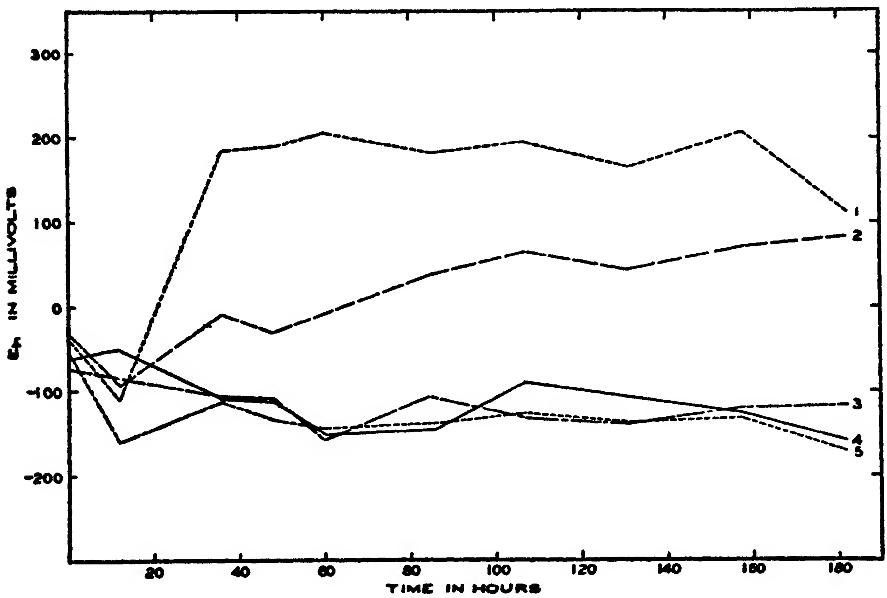

FIG. 1. EFFECT OF THE ADDITION OF NITROGENOUS AND CARBOHYDRATE MATERIALS ON THE POTENTIALS DEVELOPED IN SOIL

1, casein; 2, alanine; 3, dextrose; 4, control; 5, starch

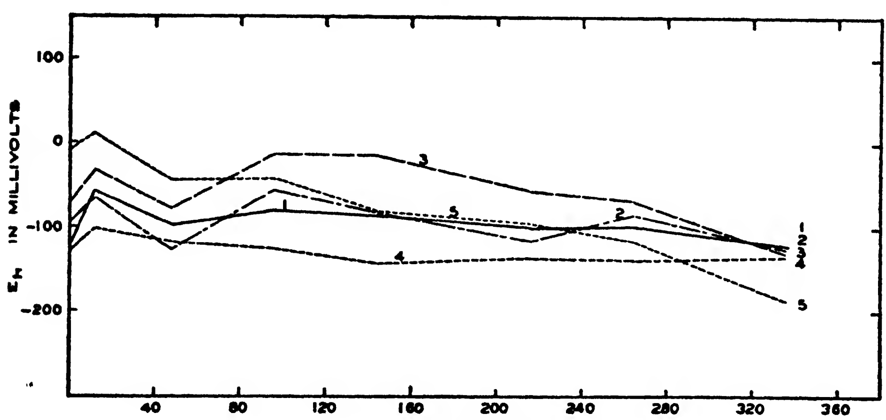

FIG. 2. EFFECT OF THE ADDITION OF COMPLEX ORGANIC MATTER ON THE POTENTIAL OF SOIL

1, stable manure; 2, rye straw; 3, fresh cut grass; 4, control; 5, rye straw + $(NH_4)_2HPO_4$

Samples of approximately 10 gm. were taken from time to time from the pots with a spatula or in a column removed with a large cork borer (the

method used appeared to make little difference). The samples were placed in 100-cc. beakers. When the sample was removed with a spatula, some packing in the beaker was necessary, but when the cork borer was used, the

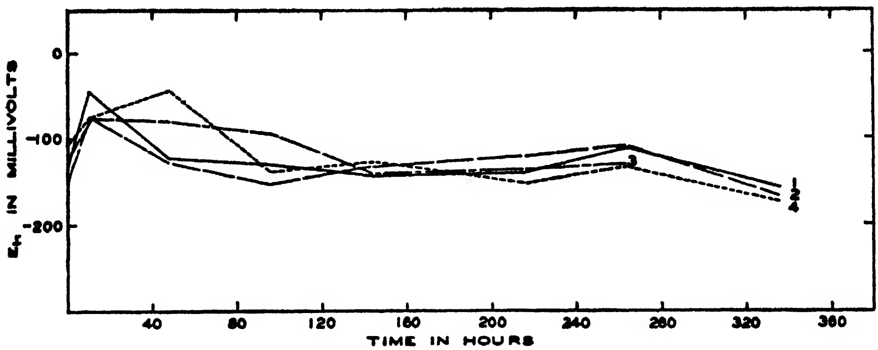

FIG. 3. EFFECT OF MOISTURE ON THE POTENTIAL OF STANDING SOIL
1, 24 per cent moisture; 2, 36 per cent moisture; 3, 18 per cent moisture; 4, 30 per cent moisture

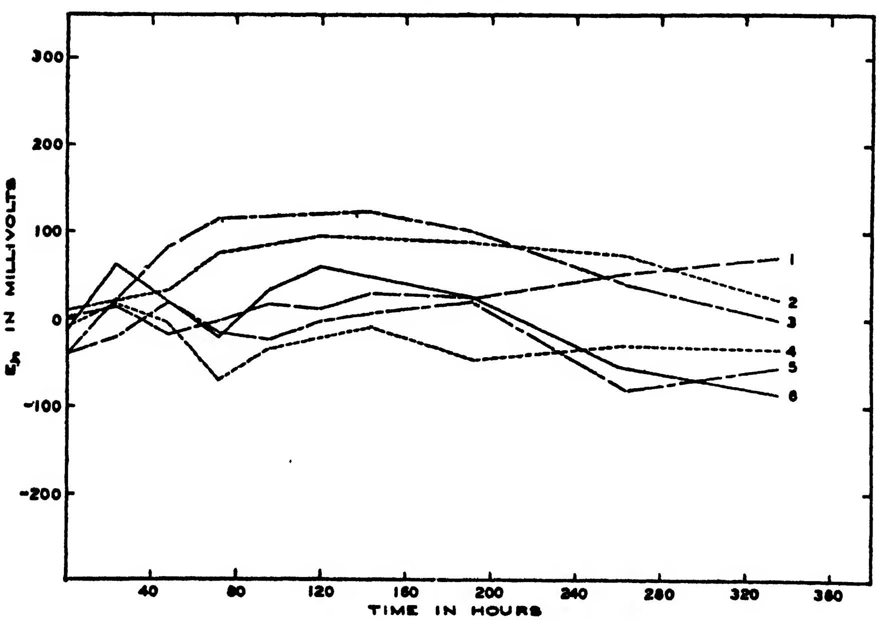

FIG. 4. SAND MEDIUM WITH A CARBOHYDRATE BASE
1, *Actinomyces californicus;* 2, *A. bobili;* 3, *Trichoderma* sp.; 4, *Bacterium fluorescens;* 5, *B. cereus;* 6, *Mucor* sp.

column of soil was expelled into the beaker without unnecessary disturbance, and, therefore, no packing was needed. After the potential measurements had been made, the soil samples were suspended in 50 cc. of distilled water,

the suspensions were allowed to stand for 2 hours, and the pH was determined by means of the glass electrode.

Part I. A quantity of air-dried soil was divided into 10 portions of 1500 gm. each. Two portions received 0.5 per cent casein; two portions, 0.5 per cent alanine; two, 0.5 per cent starch and 0.1 per cent $(NH_4)_2HPO_4$; two, 0.5 per cent dextrose and 0.1 per cent $(NH_4)_2HPO_4$; and two portions were left as controls. After thorough mixing, the soil and added ingredients were transferred to individual pots, and sufficient water was added to bring the moisture content to 20 per cent. E_h and pH measurements were made on samples removed from time to time. The results, corrected to pH 7.0, appear in figure 1.

Part II. Ten portions of 1000 gm. each of air-dried soil were used. Of these, two were unaltered; two contained 1 per cent by weight of rye straw; two, 1 per cent rye straw and

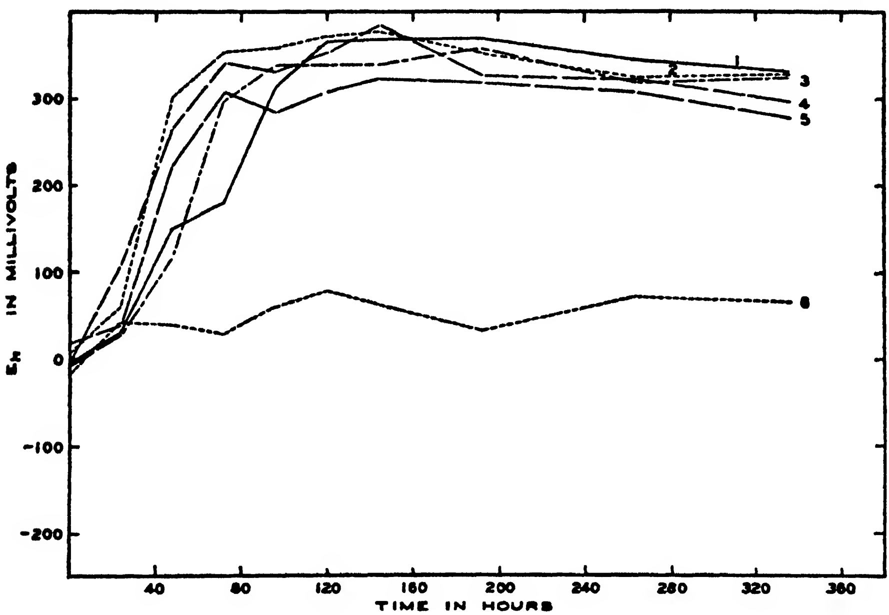

FIG. 5. SAND MEDIUM WITH A PROTEIN BASE

1, *Actinomyces californicus;* 2, *Bacterium cereus;* 3, *Actinomyces bobili;* 4, *Mucor* sp.; 5, *Trichoderma* sp.; (6), *Bacterium fluorescens*

0.1 per cent $(NH_4)_2HPO_4$; two, 4 per cent fresh cut grass; and two, 4 per cent fresh stable manure. The soils were distributed in individual pots, and water was added to bring the moisture content to 24 per cent. Potential changes in these mixtures over the period of observation are shown in figure 2.

Part III. Ten 1000-gm. portions of soil were distributed in individual pots, and water was added to bring the moisture content of pairs to 12 per cent, 18 per cent, 24 per cent, 30 per cent, and 36 per cent respectively. Changes in potential under these varying conditions of moisture are shown in figure 3.

Part IV. Two sand media were prepared; one with a protein base, 1 per cent casein, and 0.01 per cent K_2HPO_4, and the other with a carbohydrate base, 1 per cent dextrose, together with 0.02 per cent $NaNO_3$ and 0.01 per cent K_2HPO_4. Two hundred gram quantities of the sterile media in wide-mouthed Erlenmeyer flasks were inoculated with pure cultures

TABLE 1

Changes in potential as a result of different soil treatment

E_h calculated to pH 7.0, using the factor 0.060 volt per unit pH

Soil Media—Unsterilized

	POT* NUMBER		TIME IN HOURS									
			0	12	36	48	60	85	107	131	158	182
20 per cent moisture	1	Control	−65	−53	−112	−113	−155	−149	−93	−162	−129	−160
	2	Casein	−42	−113	+181	+187	+204	+180	+192	+163	+204	+109
	3	Alanine	−33	−94	−1	−31	−13	+38	+64	+43	+71	+83
	4	Starch + $(NH_4)_2HPO_4$	−53	−161	−111	−134	−144	−138	−126	−136	−131	−152
	5	Dextrose + $(NH_4)_2HPO_4$	−74	−85	−106	−109	−159	−107	−131	−136	−120	−116
			0	12	48	96	144	216	264	336	552	
24 per cent moisture	6	Rye straw	−94	−65	−126	−54	−81	−113	−82	−124	−88	
	7	Rye straw + $(NH_4)_2HPO_4$	−8	+13	−43	−41	−81	−93	−114	−185	−136	
	8	Fresh cut grass	−71	−33	−79	−13	−14	−56	−66	−130	−148	
	9	Fresh stable manure	−124	−57	−98	−79	−84	−99	−96	−121	−97	
	10	Control	−130	−102	−119	−126	−143	−136	−138	−132	−108	
	11	12 per cent moisture	−140	−142								
	12	18 per cent moisture	−151	−76	−80	−95	−141	−137	−130			
	13	24 per cent moisture	−133	−45	−123	−131	−142	−140	−111	−158		
	14	30 per cent moisture	−125	−74	−43	−139	−127	−152	−134	−174	−117	
	15	36 per cent moisture	−106	−75	−125	−151	−131	−147	−108	−168	−111	
		Sand Media—Sterilized										
			0	24	48	72	96	120	144	192	264	336
20 per cent moisture	16	Glucose—*Bacterium cereus*	−40	−21	+19	−18	−23	−2	+7	+22	−81	−55
	17	Glucose—*B. fluorescens*	−10	+20	−6	−71	−35	−10	−10	−46	−30	−35
	18	Glucose—*Trichoderma*	−41	+24	+84	+116	+118	+119	+124	+101	+41	0
	19	Glucose—*Mucor*	−15	+62	+22	−21	+34	+61	+46	+24	−54	−85
	20	Glucose—*Actinomyces californicus*	−1	+14	−18	−1	+18	+12	+30	+25	+54	+72
	21	Glucose—*A. bobili*	+8	+20	+32	+76	+97	+94	+88	+99	+74	+23
	22	Casein—*Bacterium cereus*	+6	+60	+301	+354	+359	+373	+378	+353	+326	+328
	23	Casein—*B. fluorescens*	−16	+42	+40	+29	+60	+79	+64	+33	+73	+66
	24	Casein—*Trichoderma*	+16	+37	+223	+307	+283	+308	+323	+319	+308	+279
	25	Casein—*Mucor*	−3	+106	+267	+342	+331	+354	+386	+327	+324	+298
	26	Casein—*Actinomyces californicus*	−7	+30	+148	+179	+313	+366	+369	+370	+346	+332
	27	Casein—*A. bobili*	−10	+29	+115	+297	+339	+341	+339	+333	+321	+328

* Each pot number represents a pair of pots, and, therefore, each value is the average of duplicate experiments (four actual measurements) rounded to the nearest millivolt.

of soil microörganisms. At the end of the manipulation each medium contained 20 per cent moisture. The experiments were run in duplicate, i.e., each microörganism was grown in two flasks of each medium, or four flasks in all. The following microörganisms were used: *Bacterium cereus*, *B. fluorescens*, *Trichoderma* sp., *Mucor* sp., *Actinomyces californicus*, *A. bobili*. The time-potential curves of these experiments, corrected to pH 7.0, are shown in figures 4 and 5.

A summary of the results of all the foregoing experiments is given in table 1.

DISCUSSION

The error inherent in EMF measurements on poorly poised material, such as in the present experiments, is large. It is probably not so great as some of the data, as in figures 2 and 4, would seem to indicate, for here very possibly microbial species differences exert their influence. Figure 3 is a better illustration of variation due to experimental error. Species differences in oxidation-reduction potential, first pointed out by Burrows and Jordan (4) and later confirmed by Gillespie and Rettger (8) are a very real factor in the determination of the degree of reducing intensity that occurs. It must be borne in mind that in a medium such as unsterilized soil, which contains a great variety of microörganisms, addition of various kinds of decomposable organic matter will result in the predominance of a certain type of population. Thus, observed differences in potential resulting from the addition of different kinds of organic matter may, in some cases, be the result of the activity of a different microbial flora.

Although the soil media used in the present experiments are exceedingly complex, both biologically and biochemically, the results of the first portion of the work definitely indicate that a very marked difference in potential occurs when casein or alanine and dextrose or starch are undergoing decomposition in unsterilized soil. This difference is considerably greater than the experimental error and is not the result of differences in pH, since the introduction of any correction factor for the latter tends to accentuate the wideness of separation of potential levels. That this difference is real and not the result of different types of microbial flora is apparent from the results of the experiments of Part IV. When pure cultures of soil microörganisms were grown in sterile sand media the differences in potential level in the presence of carbohydrate and nitrogenous substrates became more marked than in soil media. In the former case the same species were tested on both nitrogenous and carbohydrate substrates and resulted in striking evidence of the effect of the type of nutrient on the potential produced in cultures of microorganisms. The association of a strongly positive potential level with the decomposition of casein is shown in figure 5 in which *Bact. fluorescens* alone remains at a level comparable to that attained in the carbohydrate medium. Since this organism does not decompose casein, its remaining at a lower level seems particularly indicative of an intimate relation between the protein decomposition and positive potential level.

This association of positive potential with protein decomposition does not appear to agree with the probable activity of obligate anaerobes, which is known to be favored by the conditions of the present experiments, i.e., a rather vigorous oxidation which undoubtedly results in a rapid depletion of the oxygen supply, a supply of readily available nutrient material, and an alkaline reaction. Dack and Burrows (5) have shown, however, that some of the non-sporulating obligate anaerobes do not produce the extremely negative potentials (approaching hydrogen over-voltage) that have been found in cultures of some sporulating obligate anaerobes (12). These non-sporulating forms did not differ, in respect to potential, from the usual aerobic types. It is quite possible, therefore, that obligate anaerobic bacteria may be active in some of the present experiments in spite of the positive potential levels found to exist.

In this connection it is appropriate to point out that the statement of Heintze (9) that the negative drift in potential in standing soil is an indicator of organic matter content should be modified, since it is apparent that a negative or positive drift depends on the *nature* of the organic matter.

The results shown in figure 2 are, perhaps, deserving of comment in that qualitative differences in the added decomposable material do not result in marked differences in potential level. In these cases, of course, the results are not strictly comparable, for new bacteria were added in the form of contamination, such as in stable manure, and, therefore, the microbiological population may have differed considerably from pot to pot. It is possible that real differences do exist, but the experimental work presented here is not adequate to demonstrate them. In order to determine relatively small differences such as these, if they exist, it is necessary to resort to some statistical method such as that used by Burrows and Jordan (4); otherwise, it can hardly be said that "significant" differences are present. The complex nature of the added material and of the soil, together with present lack of information, precludes further speculation in this connection.

The negligible effect of moisture content on potentials developed in soil on standing may be noted. As has already been pointed out, apparently there is no rigid relation between the exclusion of oxygen and the potential developed. This is in agreement with the conclusion reached by Heintze (9), who found that potential was no criterion of water-logging.

In the present experiments we have not had the difficulty referred to by Peech and Batjer (11) of potential drift and the necessity of aging electrodes in solutions of proper potential before measurements could be made. This problem did arise in the measurement of potentials of muds from sea bottoms,[3] but it was found at the time that this was due to inadequate cleaning of bridges between samples. Agar bridges could not be thoroughly cleaned, but when saturated KCl solution bridges were used and flushed with con-

[3] Unpublished experiments.

siderable quantities of fresh solution, in addition to careful cleaning on the outside, between samples, this difficulty was not experienced, and a series of samples, varying considerably in their potentials, could be read in any order without affecting the final result. This technique was, therefore, continued in soil experiments and was found to be entirely satisfactory.

SUMMARY

The experimental evidence presented here indicates that the type of decomposable organic matter present in the soil is a highly important factor in the determination of the degree of reducing intensity that will prevail. Decomposition of casein was found to result in strongly positive potential levels, whereas decomposition of carbohydrate produced more negative, but not extremely negative, potentials which do not appear to differ greatly from those produced in standing soil to which no organic matter was added. The effect of moisture content on potentials developed in standing soils was found to be negligible.

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THE BEHAVIOR OF LIGNIN AND HUMIC ACID PREPARATIONS TOWARD A BROMINATION TREATMENT

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Various chemical characteristics and physical properties of the so-called "humic acids" have been investigated for many years. The progress of chemistry, however, in the study of these organic complexes resulting from the decomposition of plant material has been very slow. The reasons for this are chiefly that humic matter possesses a high molecular weight, is for the most part insoluble except in alkali, is extremely resistant to breakdown into simpler derivatives, and finally, is of variable composition depending upon the source and method of preparation.

The terms "humus" and "humic acid" are vague and indefinite because of indefinite chemical characterization but must be considered in either case as referring to a group of complex substances which compose a relatively large proportion of peat or of soil organic matter. In this paper the term "humic acids" or "humic acid fraction" is used to refer to the fraction of the organic matter dissolved by dilute alkali solution under pressure and precipitated by mineral acid. The lignin or lignin degradation products present in peat or soil organic matter are contained largely, if not completely, in this particular fraction.

The object of the present investigation was to demonstrate fundamental differences or similarities in the character of derivatives of several humic acid preparations when compared with one another and when compared with a lignin compound. These materials were prepared in the same manner and were subjected to a bromination treatment which presumably by simultaneous oxidation, substitution, and addition reactions effected very drastic alterations. The resulting products were largely ether- and alcohol-soluble; this property among others permitted an examination not possible with the original substances. Crystalline derivatives were also noted as having been formed during the bromination reaction, and it was hoped that the identification of these by-products would yield further information as to the nature of the organic materials and particularly the relationship of the humic acids to lignin.

REVIEW OF LITERATURE

Previous experiments (9) on the bromination of humic acids suspended in carbon tetrachloride indicated that practically no addition occurred but that from 22.80 to 51.53 per cent

of bromine was substituted. Similarly, the principal reaction found to take place with lignin was that of substitution, with a simultaneous splitting off of methoxyl groups (5). Friedrich and Pelikan (7) distinguished differences in the character of the methoxyl groups of lignin since only a portion could be removed by bromine. When wood is treated with bromine in the presence of water, according to Fuchs (8), the lignin is gradually destroyed whereas cellulose is not attacked. Coal may absorb 30 to 36 per cent of bromine by the action of aqueous bromine (8).

Other bromination studies (6) with lignin report the formation of quinone-like substances. Klein (10, p. 326) states that in the treatment of humic acids with potassium chlorate and hydrochloric acid small amounts of chloranil (tetrachlorquinone) are found. The formation of crystalline compounds of unknown constitution resulting from the bromination of different varieties of peat has been demonstrated (4).

On the basis of the solubility of peat in a sulfurous acid solution after removal of ether-soluble substances, Oden and Lindberg (12) concluded that a low lignin content paralleled a high degree of humification. Similar results (17) were obtained by the use of a chlorine dioxide solution to distinguish lignin from humic matter, the latter being soluble only in alkalies.

Differences in the chemical behavior of soil organic matter from different soils have been shown by Shorey (16) by means of a variety of reactions. The extensive work on humus in general has very recently been reviewed in a complete manner by Waksman (18).

MATERIALS AND METHODS

Two samples of peat, one of lignin, and one of soil organic matter were used in the bromination experiments. The peats included a sawgrass variety obtained from Zellwood, Florida, and a sphagnum moss peat from Cottage Lake near Seattle, Washington. The former was partially decomposed and moderately acid, whereas the latter was extremely acid and but very slightly decomposed. The sample of lignin was kindly furnished by Dr. Max Phillips, of this bureau, who prepared the sample from corn cobs by the alkali method (13). The source of soil organic matter was a sample of Marshall silt loam obtained from the erosion experiment station near Clarinda, Iowa. The physical and chemical characteristics of this soil have previously been described in detail (11).

Each material was ground to pass a 1-mm. mesh sieve and used in an air-dry condition for all subsequent experiments.

The humic acids were prepared by treatment of 200 gm. of peat or 2,000 gm. of soil with 2 liters of a 2 per cent solution of sodium hydroxide at 130°C., or approximately 25 pounds gauge pressure. An autoclave of 1 gallon capacity was used, and the duration of the treatment was 6 hours. The reaction mixture was filtered through a Pasteur-Chamberland filter after cooling and removal from the autoclave. The filtrate was treated with an excess of hydrochloric acid, boiled for one-half hour, and again filtered. The acid precipitate was washed several times and finally electrodialyzed until free from chlorides. This material as obtained from peat or from soil corresponds to the corn cob lignin with respect to the method of preparation.

Bromination was carried out by treatment of the materials with a mixture of bromine and water at elevated temperatures in sealed glass tubes of the Carius type. The electric furnace used for heating was fitted with four iron

pipes in which the glass tubes were placed during treatment. An ordinary thermometer was used to indicate the temperature. The proportions of bromine and water, the temperature, and the time of treatment were varied during the course of the experiments.

The tubes containing the reaction mixtures were placed in the furnace at the desired temperature and at the completion of the treatment were left overnight to cool before being opened. Pressure was usually developed and was relieved by breaking off the capillary seal. The contents of each tube were transferred to a beaker with the aid of a stream of wash water. The diluted mixture in each case was filtered through an ordinary filter paper, thereby separating the soluble and insoluble fractions.

Bromine determinations were made by a modification of the Carius method. Digestion of the material with fuming nitric acid and conversion of the halogen to silver bromide were accomplished in small glass tubes heated to 300°C., in a special electric furnace recently described by Clark (1). The silver bromide was filtered by means of a Pregl tube and weighed in the usual manner.

Ether and alcohol extracts of the brominated materials were made by means of a Soxhlet extraction apparatus. Total nitrogen was determined by the Kjeldahl method, and ammoniacal nitrogen, by distillation with magnesium oxide.

The separation and the recrystallization of crystalline material resulting from bromination were carried out as described later in connection with the identification. Molecular weight determinations were made by the boiling method with acetone as the solvent. The apparatus used was that described by Lassar-Cohn (3, p. 322).

EFFECT OF VARIATION IN TEMPERATURE AND TIME OF TREATMENT OF PEAT WITH AQUEOUS BROMINE

The effect of a variation from 1 to 4 hours' heating with an excess of bromine at 100°, 125°, and 150°C. was investigated. One-gram samples of sawgrass peat were used with 10 cc. of water and 2 cc. of bromine. It was found necessary, however, to increase the quantity of bromine to 5 cc. for the treatment at 150°C., in order to maintain an excess.

The proportions of oven-dried soluble and insoluble matter were expressed as percentages calculated on the basis of the oven-dry weight of the original sample taken for treatment. The contents of bromine and nitrogen were expressed as percentages of the particular fractions analyzed.

As shown in table 1, an increased amount of material was found to have been rendered soluble by the bromination reaction with increased time of treatment at 100°C. At higher temperatures smaller differences were found in the percentages, with a slight tendency for the quantity of soluble matter to decrease with longer periods of heating. Continued heating at any one of the temperatures employed caused a marked reduction in the percentage of the insoluble fraction. This, however, did not cause a corresponding increase

in the soluble fraction, since a portion of the material was completely oxidized to carbon dioxide or other gaseous products. Complete oxidation of a portion of the organic matter was favored either by raising the temperature or by increasing the time of treatment as shown by the percentages of total yield of the brominated product. After 1 hour of treatment at 100°C., the total yield was 140.2 per cent, whereas after 4 hours' heating at 150°C., the corresponding value was only 94.9 per cent. In the latter case the loss of material by oxidation exceeded the weight of bromine absorbed by the reaction. Considerable pressure, also, was developed in the tubes during the treatment at 150°C. Nevertheless, the resistance of peat to the action of such a powerful oxidizing agent as bromine is worthy of note.

TABLE 1

Effect of variations in temperature and time of heating on a bromination reaction with sawgrass peat*

TEMPERATURE	TIME OF TREATMENT	MATERIAL DISSOLVED BY BROMINATION	MATERIAL REMAINING INSOLUBLE	TOTAL YIELD OF BROMINATED PRODUCT	BROMINE CONTENT		NITROGEN CONTENT		
					Of soluble matter	Of insoluble matter	Of soluble matter	Of insoluble matter	NH_3-N of soluble matter
°C.	*hours*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
100	1	53.1	87.1	140.2	36.9	39.5	4.00	1 30	
	2	54.8	84.8	139.6	37.1	39.9	4 05	1.25	
	3	57.3	75.2	132.5	38.9	44 5	4.54	0.89	
	4	59.5	70.9	130.4	40.7	47.7	4.56	0 75	
125	1	58.0	74.6	132.6	41.6	47.1	4.64	0.87	
	2	56.4	76.4	132.8	41.8	45.3	4.80	0 85	
	3	57.7	58.5	116.2	47.2	49.6	5 26	0 55	
	4	54.5	54.5	108.8	49.4	52.6	5.67	0.40	
150	1	54.7	58.3	113.0	50.4	62.8	5.81	0.49	3.50
	2	60.6	41.2	101.8	58.2	63.1	5 33	0.40	3.63
	3	60.0	38.5	98.5	56.8	65.8	5.79	0.22	3.85
	4	59.5	35 4	94.9	56.1	62.1	5.52	0.23	3.91

* A portion of the data in this table has previously been reported in a doctor's thesis (4).

The total content of bromine of both the soluble and insoluble fractions increased with increasing temperature and time of treatment except after continued heating at 150°C. The percentage of nitrogen, on the other hand, steadily diminished under the same conditions until only 0.23 per cent nitrogen remained in the insoluble fraction after 4 hours' treatment at 150°C. The untreated peat contained 3.37 per cent nitrogen, and on the basis of the original content approximately 97 per cent of the total was finally rendered soluble. Ammoniacal nitrogen, determined in the soluble fraction from treatment at 150°C., was found to comprise the major portion of the total nitrogen in this fraction. Continued treatment at 150°C., however, brought about only a relatively small increase in the percentage of ammoniacal nitrogen.

BROMINATION AND FRACTIONATION OF LIGNIN AND HUMIC ACIDS

In the extraction of the alkali-soluble material from the various samples for the preparation of the humic acid fraction it was found that 71.0 per cent of sawgrass peat was dissolved by 2 per cent sodium hydroxide but that only 55.5 per cent of sphagnum peat was rendered soluble (table 2). Addition of hydrochloric acid caused a portion of the alkali extract to precipitate but in varying proportions depending upon the source of the material. Lignin, of course, was entirely alkali-soluble and acid-insoluble because of the particular method of preparation. Only 24.7 per cent of the alkali extract from sphagnum peat was recovered as acid-insoluble (humic acids), whereas 54.2 per cent was obtained in the case of sawgrass peat.

TABLE 2

Solubility of various organic materials in 2 per cent NaOH and in dilute HCl

SAMPLE	SOLUBLE IN 2 PER CENT NaOH	PROPORTION OF NaOH-SOLUBLE MATERIAL INSOLUBLE IN HCl	YIELD OF HUMIC ACID BASED ON ENTIRE SAMPLE
	per cent	*per cent*	*per cent*
Sawgrass peat	71 0	54 2	38.5
Sphagnum peat	55 5	24 7	13.7
Soil organic matter	. .	28 8	.
Corn cob lignin	100 0	100 0	100 0

TABLE 3

Fractionation of lignin and humic acids treated with bromine and water 3 hours at 110°C.

SOURCE	MATERIAL* DISSOLVED BY BROMINATION TREATMENT	MATERIAL* REMAINING INSOLUBLE	TOTAL YIELD† OF BROMINATED PRODUCT	INSOLUBLE FRACTION FROM BROMINATION: Ether-soluble	INSOLUBLE FRACTION FROM BROMINATION: Alcohol-soluble	INSOLUBLE FRACTION FROM BROMINATION: Residue	YIELD OF TETRA-BROMQUINONE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Corn cob lignin	25.5	74 5	146	90 3	9 6	0 0	10.4
Sphagnum peat	27 0	73.0	152	48 9	44 8	1 0	5.9
Sawgrass peat	30 6	69.4	154	39 8	32.7	23.8	4.2
Soil organic matter	53 3	46 7	121	28 9	39.8	31.3	1 0

* Expressed as percentage of the entire brominated product.

† Expressed as percentage of original sample taken for treatment.

The proportion of soil organic matter soluble in sodium hydroxide was not obtained, since the total content of organic matter in the soil sample was not determined. The yield of humic acids relative to the total alkali-soluble matter, however, was calculated after correction for inorganic salts in the acid filtrate from the humic acid precipitation. The latter was accomplished by treating the evaporated acid-soluble material with 30 per cent hydrogen peroxide and weighing the dried inorganic residue.

Treatment of the hydrochloric-acid-insoluble material or humic acid frac-

tion with bromine and water for 3 hours at 110°C., resulted in a product of which a large proportion remained insoluble in the reaction mixture. Considerable variation, however, was found in the relative quantities of insoluble matter after bromination of the different materials. The values ranged from 46.7 per cent in the case of humic acids from soil organic matter to 74.5 per cent in the case of corn cob lignin (table 3). The quantities of the soluble fraction varied similarly but in the reverse order.

The materials were arranged in table 3, as well as in the other tables, in the order of their degree of decomposition as found in their original or natural condition. This arrangement permits relationships to be seen more readily. Soil organic matter was considered as having been decomposed to the greatest extent, whereas corn cobs from which the lignin was isolated had undergone no decomposition. The peats were in an intermediate stage of decomposition.

The quantities of ether-soluble matter produced by bromination exhibited a regular decrease with increasing degree of decomposition with an accompanying but less regular increase in the alcohol-soluble material. There was also an increase in the material not soluble either in ether or in alcohol, amounting to as much as 31.3 per cent of the insoluble fraction from brominated soil humic acids.

SEPARATION AND IDENTIFICATION OF TETRABROMQUINONE

It was noted that the mixtures resulting from the bromination of lignin and humic acids contained a quantity of brilliant yellow platy crystals. The optimum conditions of formation appeared to be at temperatures of 105° to 110°C., with 25 cc. of water and 3 cc. of bromine per gram of sample. The time of treatment necessary was approximately 3 hours.

The crystalline material was not soluble in water and hence remained in the insoluble fraction. It was dissolved by ether, and when the evaporated ether extract was taken up with a small volume of 95 per cent alcohol the crystalline compound remained largely insoluble whereas the remaining material was dissolved. The crystals, however, were appreciably soluble in hot alcohol, which served as the solvent for their recrystallization. Those from lignin were obtained in the largest quantities and were capable of being purified the most readily.

The molecular weight, melting point, and elementary composition were determined. These values corresponded, within limits of experimental error, with values calculated (table 4) or obtained experimentally from pure tetrabromquinone. The latter was prepared for comparison from quinone by the method of Sarauw (15). Briefly, this consisted of the addition of an excess of bromine to a solution of quinone in glacial acetic acid. A good yield of tetrabromquinone separated out after the mixture had been kept hot for an hour or more. Recrystallization was accomplished in the same manner as with the compound obtained from lignin.

The melting point of both compounds, after several recrystallizations, was

found to be 295°C. (uncorrected). The total bromine content was 75.5 and 75.4 per cent, respectively, for the lignin compound and for the prepared tetrabromquinone. Crystals obtained from the peat humic acids, although possessing similar bromine contents, could not readily be purified to the extent that agreement in melting point was obtained. Optical examination by Mr. G. L. Keenan of the Food and Drug Administration, however, showed that the crystals from peat had indexes of refraction corresponding with those of the known compound.

Another property which served in confirming the identification of the crystals from lignin and humic acids as tetrabromquinone included the formation of a purple color when the compounds were dissolved in a warm solution of potassium hydroxide.

The yield of crystals from soil humic acids was very small, and no attempt was made to purify them sufficiently for analysis. They had the same characteristic appearance and gave the color test with potassium hydroxide, which left no doubt as to their identity.

TABLE 4

Analysis of tetrabromquinone as isolated from brominated lignin

CHARACTERISTIC	LIGNIN COMPOUND	CALCULATED FOR TETRABROMQUINONE
Carbon content.......................... .. *per cent*	17 63	16 98
Hydrogen content .. . . *per cent*	0 18	0.00
Bromine content... . .. . *per cent*	75 50	75 45
Molecular weight	415*	423 7
Melting point... ....°*C.*	295	298†

* Average of four determinations.

† As reported by Datta and Chaterjee (3).

The crystalline material as obtained from each sample was weighed in the impure condition before the first recrystallization, and the percentage yield was calculated on the basis of the weight of the entire brominated sample. These results, although not highly accurate, are comparative and serve to distinguish the different types of organic matter. The percentages (included in table 3) show the greatest yield for lignin and the least for soil humic acids.

Oxalic acid was found in the soluble fractions from bromination, but since this compound is commonly produced in oxidation reactions no conclusions could be drawn from the formation of this substance relative to differences in character of the humic acids.

BROMINE CONTENT OF VARIOUS FRACTIONS OF BROMINATED LIGNIN AND HUMIC MATERIAL

The bromine content of the insoluble fraction from the bromination treatment was greatest in the case of lignin and least in the humic acids from soil organic matter as shown in table 5. The reverse was true of the soluble

fraction, although smaller differences were noted in the analyses of the latter fractions.

In order to obtain some information relative to the nature of the combined bromine, small samples of the insoluble fraction (0.2 gm.) were boiled 5 minutes with 15–20 cc. of a 5 per cent solution of sodium hydroxide in absolute alcohol. The mixtures were then diluted to approximately 75 cc. with water, acidified with nitric acid, and filtered to remove all precipitated organic matter. Silver nitrate was then added, and the silver bromide was determined in

TABLE 5

Bromine content of various fractions of brominated lignin and humic acids

SOURCE	INSOLUBLE FRACTION FROM BROMINATION			TOTAL Br OF SOLUBLE FRACTION FROM BROMINATION
	Total Br	Br split off by alcoholic NaOH	Proportion of total Br split off by alcoholic NaOH	
	per cent	*per cent*	*per cent*	*per cent*
Corn cob lignin	64.0	38 2	59.7	43.5
Sphagnum peat	54.8	41 7	76 0	37 5
Sawgrass peat	53.4	36 2	67.8	44 7
Soil organic matter	40.0	34 4	86 0	47.7

TABLE 6

Nitrogen content of various fractions of brominated lignin and humic acids

SOURCE	N CONTENT OF UNTREATED HUMIC ACID	INSOLUBLE FRACTION FROM BROMINATION			SOLUBLE FRACTION FROM BROMINATION			
		Total N	Ether-soluble N	Alcohol-soluble N	Total N	Total N calculated as proportion of original N	NH_3-N	NH_3-N calculated as proportion of soluble N
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Corn cob lignin	1.80	0 38	0 50	2 63	4.15	86.1	1 65	39.8
Sphagnum peat	2.39	0 98	0 54	1 62	3 80	65 2	1 51	39.7
Sawgrass peat	3.92	1 62	0.95	2.70	4.86	58 4	1 95	40.1
Soil organic matter	4.90	2 12	1.06	2 45	5 76	75 8	2 68	46 5

the usual manner. Only the readily ionizable bromine is obtained by this method, since that linked to an aromatic nucleus is not split off by the alcoholic sodium hydroxide. The results as recorded in table 5 indicate that nearly 60 per cent of the bromine in the brominated lignin is split off as compared with 86 per cent of the brominated soil humic acids.

NITROGEN DISTRIBUTION

The nitrogen contents of the untreated lignin and humic acid fractions were quite different, as shown in table 6. The humic acids from soil organic mat-

ter contained nearly 5 per cent nitrogen, whereas humic acids from the less decomposed materials contained less nitrogen. Lignin contained 1.80 per cent nitrogen, the least of any of the materials.

Most of the nitrogen of the different materials was rendered soluble by the bromination treatment; to this extent there was some degree of similarity in the behavior of the nitrogenous constituents. The proportional amounts of the total nitrogen which were dissolved varied from 58.4 per cent for the humic acid fraction from sawgrass peat to 86.1 per cent for lignin. The corresponding percentage for soil humic acids was 75.8, which was not greatly different from the value for lignin.

The quantities of nitrogen remaining insoluble possessed the same relative order of magnitude as the nitrogen contents of the untreated materials (table 6). This relationship held also for the nitrogen of the ether extracts. The alcohol-soluble materials contained irregular percentages of nitrogen.

From 39.7 to 46.5 per cent of the nitrogen found in the soluble fraction was ammoniacal in form. This variation, however, was not sufficiently wide to permit definite distinctions to be made between the different materials relative to the character of the soluble nitrogen.

SUMMARY AND CONCLUSIONS

The lignin and humic acid preparations studied in this investigation had similar properties to the extent that they were each soluble in sodium hydroxide solution and were precipitated by dilute hydrochloric acid. The bromination treatment, however, has served to distinguish sharply not only between the lignin as compared with the humic acids but also among the individual humic acid fractions.

The conditions of bromination were arbitrarily fixed as to quantity of bromine and water, temperature, and time of treatment. A variable product results from the treatment under varying conditions as shown in the case of a sample of peat.

Differences in character of the different preparations were most clearly and consistently illustrated by the proportions of organic matter dissolved during the bromination treatment, by the relative quantities of ether-soluble matter produced, and by the relative yields of crystalline tetrabromquinone. The comparative ease with which combined bromine is removed by treatment with alcoholic sodium hydroxide was also shown to be a distinguishing characteristic.

The untreated lignin and humic acids contained widely different percentages of nitrogen, but some degree of similarity was shown in the proportions of the total nitrogen dissolved during the bromination treatment and in the character of the soluble nitrogen with respect to the ammoniacal form. The percentages of insoluble nitrogen, however, exhibited variations in the same relative order as did the nitrogen contents of the untreated samples.

Evidence of the presence of aromatic constituents in the composition of

lignin, and to a lesser degree in the humic acid preparations, was found in the production of tetrabromquinone. The presence of aromatic groups is in accord with the majority of formulas proposed for lignin (14).

The chemical behavior of the humic acid preparations toward bromination appeared to vary according to the degree of decomposition of the material from which these fractions were separated. Comparisons with the behavior of lignin indicated, in general, that the humic acid fraction becomes less similar to lignin as decomposition advances. It is of course recognized that lignin from different sources varies in composition and that other comparisons might have shown somewhat different relationships.

If the formation of tetrabromquinone is at all characteristic of lignin under the conditions of the bromination treatment it may be concluded from the relative yields as well as from the other relationships already discussed that lignin comprises only a small part of the humic acid fraction derived from partly decomposed peat or soil organic matter. Lignin, if the type found in corn cobs was originally present in the plant material, must, therefore, either have undergone extensive decomposition or have lost its identity in the formation of new complexes (19) which comprise the humic acid fraction.

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RECLAMATION OF ALKALI SOILS BY ELECTRODIALYSIS

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The application of the principle of electrodialysis to the removal of exchangeable bases from soils is well known. It is based on the fact that soil particles, being negatively charged, move toward the anode, and exchangeable bases, toward the cathode. If the particles in a wet soil block are not free to move, then it is the water that moves, and we have the phenomenon of electrosmosis. As a matter of fact in soils we are dealing both with electrodialysis and electrosmosis.

It is well known that alkali soils owe their characteristic features to the presence of exchangeable sodium, and improvement in such soils is accompanied by a marked reduction in the latter. The underlying principle of all the methods of reclaiming alkali soils is the removal of exchangeable sodium, which can be brought about by treatment with a Ca salt or an acid.

The application of electrodialysis on a field scale is a novel method which holds vast possibilities. Not only are no extraneous chemicals required, but the by product is sodium hydroxide, which is of economic value. A very important feature of the process is the increased rate of percolation and opening up of the soil. The rate of percolation increases as soon as the electric current is switched on and continues long after it is switched off. This residual effect is of great importance and the one likely to prove most useful in reducing the cost of reclamation.

The present investigation was divided into two parts, one dealing with laboratory experiments and the other with field trials.

LABORATORY EXPERIMENTS

A large Buchner funnel 18 inches in diameter was filled with a highly alkaline soil of pH value 11. The usual porcelain sieve of the funnel was replaced by a perforated brass plate and formed the cathode. Five cylindrical anodes were arranged symmetrically at the top along the circumference. A layer of water 1.5 inches deep was maintained on top of the soil. The wet soil was absolutely impervious to water: not a drop came out in 24 hours.

A current of 1.5 amperes with a terminal potential of 20 volts was allowed to pass through the soil. After a few minutes the water began to trickle in drops and soon formed a thin stream. After 4 hours, the pH value of the water coming out was 12.62. The current was stopped; the rate of percolation decreased

but still remained appreciable. After 36 hours, the pH value of the soil in the vicinity of the anodes was 8.66, while the soil in the top center nearer the cathode was 10.70. Once again in the same soil a current of 1.5 amperes was passed for 2 hours, and the cathode liquid was collected and titrated for alkalinity. It was found to be 0.33 *N*, which became 0.4 *N* after another hour. The current was then increased to 2.4 amperes, and within an hour the normality of the percolate went up to 0.6 *N*. The current was then stopped, and the percolate was collected overnight. The alkalinity of the liquid collected next morning was 0.5 *N*. This alkali was 90 per cent NaOH and the rest Na_2CO_3. The head of water at the top of soil was maintained at about 1.5 inches by adding fresh water, and leaching went on for several days without any current. The pH values of the different portions of the soil on the second day were as follows: anodic space, 5.53; interspaces between anodes, 10.11; cathode space, 13.28.

Another experiment in which a soil of pH 10.5 was used gave an equivalent of 13 gm. of NaOH in 5 hours with a current of 2.5 amperes and a terminal potential of 10 volts. After the current was shut off, another 4 gm. of NaOH was collected in the percolate during 14 hours. The anodes in this case were plates of ordinary iron and rustless steel. They were eaten away to the extent of 50 per cent and 25 per cent respectively. Ordinarily the cost of replacing electrodes would not be a matter of serious consideration, as any scrap iron could be used and an iron salt left in an alkali soil would be rather an advantage.

Another experiment was arranged in a wood box 2 by 2 by 3 feet. Five types of cathodes, as follows, were used:

A. Iron pipe 2 feet long perforated all around.
B. Iron pipe 2 feet long perforated on the upper surface only.
C. V-shaped wood pipe 2 feet long covered with thin perforated iron sheet.
D. V-shaped wood pipe covered with perforated brass sheet.
E. Porous tube containing tubular wire-gauze as electrode.

A current of 6 amperes was run for 3 hours with a terminal potential of 33 volts. The total current consumed was 0.6 unit. Discharge of alkali was as follows:

A = 250 cc. of 0.2 *N* solution
B = 200 cc. of 0.25 *N* solution
C = 115 cc. of 0.2 *N* solution
D = 200 cc. of 0.2 *N* solution
Total NaOH 6.6 gm.

Percolation in E was very small and consequently was not recorded. Unlike the others, the percolate, however, was absolutely clear.

The current was next increased to 10.5 amperes with a terminal potential of 50 volts. The energy consumed was 1 unit. The amount of alkali collected in 2 hours was as follows:

A = 250 cc. of 0.33 N solution
B = 250 cc. of 0.36 N solution
C = 225 cc. of 0.28 N solution
D = 250 cc. of 0.3 N solution
Total NaOH = 12.7 gm.

Percolation went on during the night without any current. Next morning 700 cc. of 0.25 N alkali was collected from all the four electrodes.

A current of 14 amperes was next passed for 4 hours with a terminal potential of 66 volts. The energy consumed was 3.7 units. The following quantities of alkali solution were obtained:

A = 950 cc. of 0.33 N solution
B = 600 cc. of 0.36 N solution
C = 500 cc. of 0.33 N solution
D = 400 cc. of 0.36 N solution
Total NaOH = 34 gm.

The most important finding from the laboratory studies was the increased rate of percolation which continued even after the current was switched off.

FIELD EXPERIMENTS

A plot of land 15 by 15 feet was trenched all around. The trench was 3.5 feet deep and 1 foot wide. The cathode was an iron tube 6 feet long and 4 inches in diameter with perforations at the top. This was buried in the soil to a depth of 3 feet by digging a furrow from the center of the plot toward the middle of the trench. It had a gentle slope toward the trench to ensure easy flow of water. A ridge about 6 inches high was made all around the plot to ensure a certain head of water. The trench was filled with water, and a liberal watering was given at the top to bring about the initial saturation of the soil, after which the water from the trench was pumped out. The anode was a big sheet of iron 3 by 6 feet long laid at the top of the moist soil block just above the cathode. After the initial saturation the plot was watered to a depth of 4 inches. No attempt was made to keep the head of water constant, as the experiment did not last long enough to necessitate that precaution. A vessel was put beneath the protruding end of the cathode in the trench to find the initial rate of percolation. It was practically nil. A current of 22 amperes from a 220-volt main was passed through a suitable resistance. Alkali began to trickle almost immediately and soon formed a thin stream; 2.5 liters were collected in 45 minutes. The strength of the solution was 0.1 N. Rains intervened, and the experiment had to be discontinued. One year after, the same electrodes were connected again. During the intervening period the cathode had become choked, and, therefore, when the current was switched on the alkali did not come out until after an hour and even then the rate of percolation was only 300 cc. an hour, which fell to 50 cc. an hour when the current was switched off. The increase in the rate of percolation was very marked and was tested several times by switching the current on and off. The strength of

the alkali coming out was 0.05 *N* against 0.1 *N* obtained the previous year. The soil, however, had been considerably improved by the first treatment and the rains that followed. There was a remarkable decrease in the exchangeable sodium in the surface 3 inches. To start with it had 4.8 m.e. per 100 gm., which fell to 3.5 after 2 hours' treatment and to 2.8 on further treatment for 4 hours. The total current consumed was 5 units.

Next, a field 17 by 50 feet was selected and trenched all around. The anode was a sheet of iron; and the cathode, a twisted wire rope about 1 inch in diameter (a rejected rope of a steam tackle), was laid in the drain on three sides. The plan of the experiment is shown in figure 1.

A current of 22 amperes with a terminal potential of 153 volts was passed through. When the anode was in position *a*, the total resistance was 7 ohms; at positions *b* or *c*, it was 9 ohms. When the area of the anode was increased

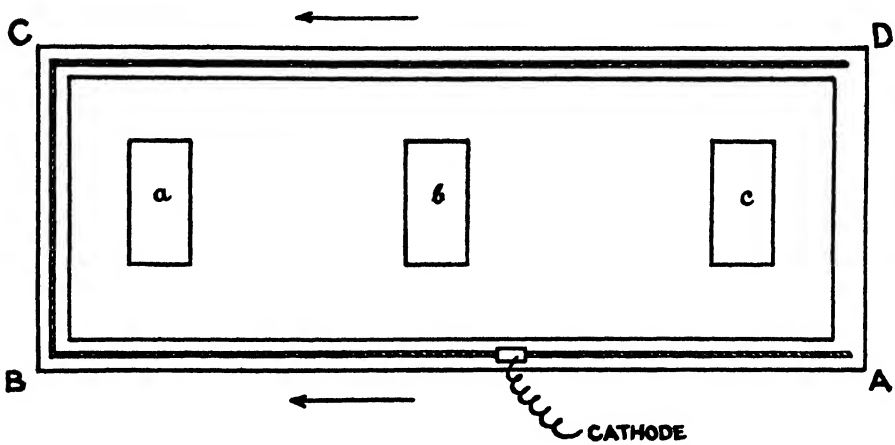

FIG. 1. ELECTRO-RECLAMATION OF SOIL—PLAN OF FIELD EXPERIMENT II

about five times, the resistance fell to 2 ohms. Increasing the area of the cathode did not have very appreciable effect on resistance.

A good deal of water was standing in the drain B-C, and in 2 hours the alkalinity of the water rose by 1 m.e. per liter. Because of a slope, there was not much water in the drains A-B and C-D. The alkalinity of a small amount of water collected in a puddle had increased by 6 m.e. per liter as a result of the passage of the electric current. The alkalinity of the water around the anode decreased by 1.3 m.e. per liter. During 5 hours' electrodialysis the soil had lost per 100 gm. 0.6 m.e. of exchangeable sodium in the top 3 inches.

DISCUSSION OF RESULTS

It is not possible at this stage to devote much space to the discussion of results. Facts are presented merely to illustrate the possibility of applying electrodialysis to the reclamation of alkali soils. Before starting these experiments, the authors had grave misgivings as to the success of the method. It

was argued that the soil would act as an infinite conductor, and the whole of the electrical energy would be diffused. Practical trials, however, proved this supposition to be incorrect. The method, it seems, holds out promise of practical importance in other directions as well. During the process of electro-reclamation, phosphates and nitrates are likely to move to the upper surface where they will prove useful to plants. This point and the effect on the micro-biological population of the soil are being studied. The application of gypsum together with electric treatment is likely to prove more beneficial than without such treatment. A preliminary experiment in this direction showed a marked decrease in electrical resistance when gypsum was applied.

The economics of electro-reclamation have not been studied thoroughly. They must depend largely on local conditions, such as the cost of electricity and the value of the land, and must be worked out by individual workers themselves. In the light of our experience, however, we can offer the following suggestions, which might prove helpful to others who may like to give a trial to electro-reclamation:

The resistance of the soil block should not exceed 1 ohm. This was accomplished in our soil with a clay content of about 15 per cent, in a plot of land 50 by 50 feet. The most important factor in this connection is the size of the anode, which can be made out of large sheets of scrap iron. Soils with a heavier texture will require a larger anode than lighter ones.

The terminal potential should be low and the amperage high. We suggest a generator of 25 volts and 250 amperes.

Electric current should not be passed continuously for a long time. It is more advantageous to pass the current for 5 to 6 hours and then allow an interval of about the same time or longer.

SUMMARY

The well-known principle of electrodialysis for the removal of exchangeable bases has been applied to the reclamation of alkali soils.

Preliminary experiments have been described which show that exchangeable sodium as NaOH can be removed from soil on a field scale by the application of electric current, resulting in a marked reduction in the exchangeable sodium of the soil thus treated.

THE EFFECT OF CALCIUM IONS AND REACTION UPON THE SOLUBILITY OF PHOSPHORUS[1]

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The literature on the subject of the fixation and availability of phosphorus is voluminous though frequently contradictory. The synonymous use of "availability" and "solubility" has led to much confusion. Statements made concerning the tendency of a cation to precipitate phosphorus from solution without specifying the existing pH or the other cations or anions present have conflicted with statements on the same subject by other investigators working under slightly different conditions.

No attempt will be made in this paper to give a complete review of the literature pertaining to the subject. To do so adequately would require the compilation of an extensive bibliography.

Recent investigations of a closely related nature have been conducted by: Gaarder (4), Austin (2), Teakle (14), Patten (11), Osugi, Hoshie, and Nishigaki (9), McGeorge and Breazeale (8), Heck (6), Sanfourche and Henry (12), Ford (3), Antonov (1), and Spurway (13).

The investigation described in this paper is based largely upon the work of Gaarder (4), with some variations, however, in the nature and treatment of the systems used. The authors, as all workers in this field should be, are appreciative of Gaarder's excellent work, and in so far as the procedures used by them correspond to those of Gaarder, the results obtained corroborate his. Unquestionably the work of many others is fully as valuable as that of those listed but is perhaps not so specifically related to this investigation.

EXPERIMENTAL PROCEDURE AND RESULTS

The results reported in this paper are from a study of the solubility of phosphorus in dilute solutions of H_3PO_4 when treated with varying amounts of different calcium compounds or of a soil saturated with calcium, as the pH of the systems was varied by different means. The mixtures, contained in stoppered bottles of suitable size, were agitated continuously for 24 hours in a water bath at 25°C. At the end of the agitation period the contents of the bottles were filtered as rapidly as possible, and the amounts of calcium and phosphorus in the filtrates and their pH values were determined by standard methods. Water free from CO_2 was used throughout the work.

[1] Contribution No. 203 from the department of chemistry. Data from a thesis to be presented as partial fulfilment of the requirements for the degree of doctor of philosophy. Published with the consent of the Graduate Council.

TABLE 1

Effect of increasing amounts of $CaCO_3$ upon phosphorus solubility in a dilute solution of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN SOLUTION	P IN SOLUTION
	gm.		*gm.*	*gm.*
1	0.0511	4.88	0.0511	0.0791
2	0 1021	5.39	0.0536	0.0791
3	0.1532	5.52	0.0538	0.0785
8†	0.7660	5.89	0.0590	0.0782
9	1.1490	6.59	0.0345	0.0184
10	1.5320	6 62	0.0361	0.0092
14	4.5960	7.01	0.0401	0.0064

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† Several points were established between bottles 8 and 9. The data were destroyed by fire and were not repeated since they showed nothing unusual.

TABLE 2

Effect of a constant amount of calcium ion, added in varying proportions of $CaCO_3$ and CaO, upon phosphorus solubility in dilute solutions of H_3PO_4

BOTTLE NUMBER	Ca AS CaO*	Ca AS $CaCO_3$	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.	*gm.*		*gm.*	*gm.*
1	0.0000	0 3064	5.67	0 0548	0 0786
2	0 0511	0 2554	6 59	0 0264	0 0185
3	0.1021	0.2043	7.08	0.0227	0 0013
4	0.1532	0 1532	7.36	0 0011	0.0000
5	0 2043	0 1021	11.05	0 0259	0 0000
6	0 2554	0 0511	11.78	0 0529	0 0000
7	0.3064	0 0000	12.14	0 0781	0 0000

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

TABLE 3

Effect of a constant amount of $CaCO_3$ (containing 0.3064 gm. of Ca) upon phosphorus solubility in dilute solutions of H_3PO_4 when the reaction of the system was varied with NaOH or HCl*

BOTTLE NUMBER	0.15 *N* HCl ADDED	0.3 *N* NaOH ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.	*cc.*		*gm.*	*gm.*
2	100	0	2.28	0.3064	0.0791
3	75	0	5.09	0.2814	0.0778
4	50	0	5.36	0.2087	0.0779
5	25	0	5.51	0.1324	0.0781
8	0	0	5.79	0.0563	0.0779
9	0	1.25	6.06	0.0517	0.0704
10	0	2.50	7.14	0.0185	0.0108
13	0	10 00	7.84	0.0018	0.0117
14	0	20.00	10.04	0.0000	0 0359
15	0	30.00	11.21	0.0000	0.0188

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

The pH values below neutrality were determined by the quinhydrone electrode, and those above, by the hydrogen electrode. Calcium and phosphorus were determined by methods approved by the Association of Official Agricultural Chemists. Phosphorus was determined colorimetrically in some of the filtrates which yielded no precipitate of ammonium phosphomolybdate. Less than 0.5 p.p.m. was found in such cases. In most instances the amount in

TABLE 4

Effect of increasing amounts of CaO upon phosphorus solubility in dilute solutions of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.		*gm.*	*gm.*
2	0 0511	4.36	0 0511	0.0791
3	0 0574	5.50	0 0552	0 0784
4	0 0638	5 35	0 0462	0 0709
6	0 1021	5 47	0 0259	0.0409
7	0 1276	5 50	0 0137	0 0235
8	0 1404	5 92	0 0085	0 0135
9	0 1532	5 94	0 0039	0 0043
18	0 3064	12 21	0 0781	0 0000

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

TABLE 5

Effect of a constant amount of $CaCl_2$ (containing 0 2043 gm of Ca) upon phosphorus solubility in dilute solutions of H_3PO_4, when the reaction of the system was varied with NaOH*

BOTTLE NUMBER	0.15 *N* NaOH ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.		*gm.*	*gm.*
6	9 0	5 09	0 2043	0 0791
7	9 5	5 05	0 1978	0 0749
8	10 0	4 82	0 1889	0 0706
9	12 5	5.07	0 1568	0 0408
10	15 0	5 38	0 1431	0 0403
11	20 0	5 44	0 0955	0 0188
12	25 0	5 48	0 0528	0 0024
13	26 0	5 73	0 0415	0 0005
14	28 0	5 91	0 0320	0 0001
15	29.0	7 36	0 0263	0 0000
20	75 0	12 43	0 0018	0 0000

* Added to 250 cc. of solution containing 0 0791 gm. of P as H_3PO_4.

250 cc. of filtrate was less than 0.1 mgm.; hence, solubility in all such cases has been listed merely as 0.0000 gm.

Throughout the work, unless an exception is cited, each addition of calcium was made to 0.25 gm. of H_3PO_4 (0.0791 gm. of P) diluted to 250 cc. Likewise the amounts listed in the tables are based upon 250 cc. of filtrate. Certain insignificant data were omitted for the sake of brevity, as indicated by bottle numbers missing from the tables.

In the tables are summarized the nature and amounts of substances added and the results obtained. Graphical representations of the results appear in figures 1, 2, and 3.

The failure of $CaCO_3$ to precipitate the phosphorus completely, as indicated in table 1 and in figures 1 and 2, appeared to be due either to its slight solubility or to its moderate effect upon the reaction, or both. This suggested its use with CaO (table 2, fig. 2) and with NaOH (table 3, fig. 2), in which

TABLE 6

Effect of increasing amounts of a soil saturated with calcium upon phosphorus solubility in dilute solution of H_3PO_4

BOTTLE NUMBER	Ca ADDED*	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	gm.		gm.	gm.
1	0.0511	3.21	0.0301	0.0632
2	0.1021	3.99	0.0336	0.0586
3	0.1532	4.53	0 0329	0.0548
4	0.2043	4.84	0.0305	0 0495
5	0 2553	4.99	0.0281	0 0453
6	0 3064	5.27	0.0253	0 0406
7	0 3575	5.37	0 0246	0 0363
8	0 4085	5 50	0 0224	0 0324
9	0 4596	5 60	0 0213	0 0292
10	0.6128	6 32	0 0177	0 0185
11	0 7660	6 81	0 0175	0 0133
12	0 9192	7 06	0 0169	0 0098
13†	0.6128	6 48	0 0213	0 0138
14	0 7660	6.72	0 0318	0 0138
15	1 0724	6 85	0 0388	0 0075
16	1 2256	6.98	0.0375	0.0063
17	1 3788	7.02	0 0375	0 0038
18	1.5320	7.12	0 0350	0.0025

* Calculated as replaceable Ca^{++} and added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† Beginning of greater dilution. Added to 2500 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

case the amount of calcium in solution could be varied or it could be kept constant and the reaction varied. CO_2 released from the $CaCO_3$ by the action of H_3PO_4 appeared to exert a marked effect upon the results in the foregoing experiments; hence CaO was also used alone (table 4, figs. 1 and 2). Since CaO is only moderately soluble and affects the reaction strongly, it was considered of interest to try the effect of $CaCl_2$, a freely-soluble though neutral salt. The results of this experiment are not tabulated here; however, it is significant to note that calcium added in this form in amounts ranging from

$\frac{1}{3}$ to 30 times the amount necessary to form $Ca_3(PO_4)_2$ failed to produce a precipitate, and the pH remained practically constant at slightly above 2. When the reaction of such systems was varied with NaOH, however, very

TABLE 7

Effect of a constant amount of soil saturated with calcium (containing 0.2553 gm. of replaceable calcium) upon phosphorus solubility in dilute solutions of H_3PO_4, when the pH of the system is varied with HCl, NaOH, CaO, or $CaCO_3$*

BOTTLE NUMBER	HCl ADDED	0.3 *N* NaOH ADDED	CaO ADDED	$CaCO_3$ ADDED	pH OF FILTRATE	Ca IN FILTRATE	P IN FILTRATE
	cc.	*cc.*	*gm.*	*gm.*		*gm.*	*gm.*
1	20†	0	0	0	0.02	0 2519	0 0786
2	15	0	0	0	0 29	0 2511	0 0755
3	10	0	0	0	0 39	0 2457	0 0690
4	125	0	0	0	1.96	0 2236	0.0342
5	75	0	0	0	2.56	0 1885	0.0220
6	25	0	0	0	3 74	0.0892	0 0303
7	0	0	0	0	5 02	0.0275	0 0428
8	0	10	0	0	6 31	0 0073	0 0424
9	0	25	0	0	7.57	0 0051	0 0366
10	0	40	0	0	7.73	0 0031	0 0229
11	0	55	0	0	8.00	0 0031	0 0124
12	0	0	0.0357	0	5 83	0 0255	0 0349
13	0	0	0.0714	0	6 27	0 0243	0 0295
14	0	0	0.1428	0	6.68	0 0188	0 0103
15	0	0	0.2142	0	7.22	0 0133	0 0022
16	0	0	0.2856	0	7 29	0 0120	0 0020
17	0	0	0 4284	0	7.46	0 0167	0 0000
18	0	0	0 6426	0	8 91	0 0187	0 0000
19	0	0	0 8568	0	9 21	0 0379	0 0000
20	0	0	0	0.3827	6 39	0 0326	0 0408
21	0	0	0	0 7654	6 59	0 0343	0 0392
22	0	0	0	1 9135	6 62	0 0356	0 0363
23	0	0	0	3.8270	6.72	0 0359	0 0347
24	0	0	0	7.6540	6.77	0 0356	0 0306
25	0	0	0	11.4810	6.89	0 0358	0 0277
26	0	0	0	15 3080	6.93	0 0332	0 0244
27	0	0	0	19.1350	6.99	0.0360	0 0240

* Added to 250 cc. of solution containing 0.0791 gm. of P as H_3PO_4.

† In bottles 1 to 3, concentrated HCl was used; in bottles 4 to 6, 0.15 *N* HCl was used.

pronounced effects upon phosphorus solubility occurred, as shown in table 5 and figure 2. In order to determine whether the principles indicated in the preceding work would hold with the more complicated soil systems, a soil

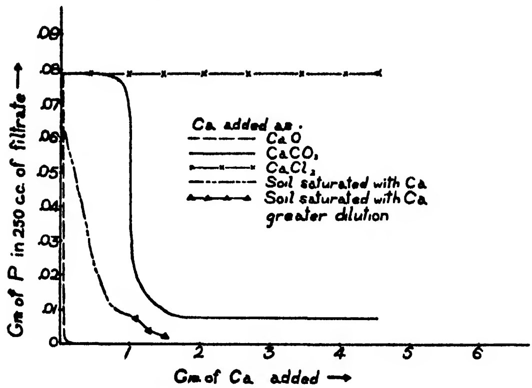

FIG. 1. EFFECT OF INCREASING AMOUNTS OF CALCIUM ION, ADDED IN DIFFERENT COMPOUNDS, UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4

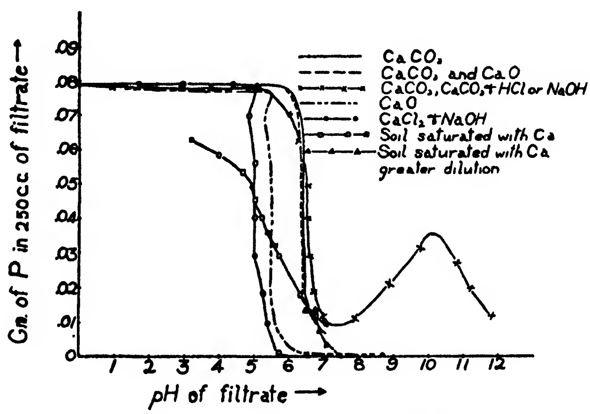

FIG. 2. EFFECT OF CALCIUM ION, ADDED IN DIFFERENT COMPOUNDS, UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4 AS THE pH IS VARIED BY THE CALCIUM COMPOUND ITSELF OR BY THE ADDITION OF ACIDS OR BASES

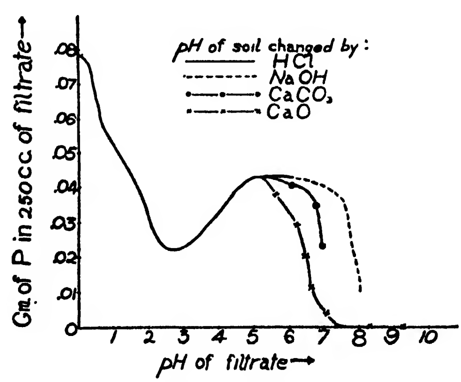

FIG. 3. EFFECT OF A CONSTANT AMOUNT OF SOIL SATURATED WITH CALCIUM UPON THE SOLUBILITY OF PHOSPHORUS IN A DILUTE SOLUTION OF H_3PO_4 WHEN THE pH OF THE SYSTEM IS CHANGED BY HCl OR VARIOUS BASES

saturated with calcium was subjected to similar treatment, both alone (table 6, fig. 2) and when the reaction was varied by HCl, NaOH, CaO, or $CaCO_3$ (table 7, fig. 3).

A quantity of Wabash loam soil was leached with normal calcium chloride solution until the leachate no longer yielded a positive test for the Mg ion. The excess $CaCl_2$ was removed by washing with distilled water. Replaceable calcium was determined by Gedroiz's method (5) and was found to comprise 20.38 m.e. per 100 gm. of soil. On the basis of replaceable calcium, sufficient soil was used to yield the different amounts of calcium listed in tables 6 and 7. In table 6, bottles 1–12 contained 0.25 gm. of H_3PO_4 in 250 cc. of solution; however, the large bulk of soil made it impossible to carry the work very far with this dilution. In order to extend the work and also to determine the effect of greater dilution upon the removal of phosphorus from solution, a dilution ten times as great was used in bottles 13–18.

DISCUSSION OF RESULTS

From the data, especially that with $CaCl_2$ alone, it is evident that Ca^{++} ions do not precipitate PO_4^{---} ions from solution, even in the presence of a high concentration of Ca^{++} ions, so long as the pH of the solution remains sufficiently low. This, of course, is a fact of importance in analytical work, as indicated by Patten's work (11). The results presented in tables 1, 2, 3, 4, and 5 and their graphical representations in figures 1 and 2 show that appreciable precipitation from these solutions did not begin until the pH approached 5.5, and maximum precipitation, or minimum solubility, did not occur until the pH approached 7.5. Although the minimum solubility of phosphorus varied considerably with the various combinations, its solubility remained almost constant at all higher pH values in the range covered, except in the work with $CaCO_3$ and NaOH, where the solubility rose somewhat, possibly because of an increase in the Na^+-ion concentration. The variations observed here are probably due to the presence of anions and cations which might be avoided in carefully controlled analytical conditions but which commonly occur in the soil.

The work with $CaCO_3$ shows that it did not reduce the phosphorus in solution to 0.0000 gm. during the 24-hour agitation period, although an enormous excess of $CaCO_3$ compared to phosphorus was present. This appears to be due to the slight solubility of the $CaCO_3$ and its moderate effect upon the pH of the solution. However, when the pH was raised with NaOH, $CaCO_3$ still did not cause complete precipitation of phosphorus, a fact which indicates that its slight solubility is a major factor in its failure to do so. This was perhaps partly due to the effect of the unlike Na^+ ions; however, there were apparently not enough Ca^{++} ions to overcome this effect and to precipitate completely the phosphorus, as occurred when soluble $CaCl_2$ was used.

It was of interest to notice that when the CO_3^{--} ion was present there was a tendency toward supersaturation of the solutions with respect to calcium phosphate. Often a short time after filtration the filtrates became cloudy

and calcium phosphate precipitated; hence, in order to obtain consistent results, it was imperative to withdraw the aliquots for analysis soon after filtering.

The work with mixtures of $CaCO_3$ and CaO and with CaO alone shows that the solubility of CaO and its effect on the pH were great enough to cause it to reduce the phosphorus content of the filtrate to 0.0000 gm. when only slightly more than enough to form $Ca_3(PO_4)_2$ with the H_3PO_4 present had been added. Figures 1 and 2 illustrate these differences. The results recorded in table 4 are of especial interest since through the use of CaO and H_3PO_4 no cations or anions other than Ca^{++} and PO_4^{---} were introduced to vary the H^+- and OH^--ion concentration.

Work with mixtures of CaO and $CaCl_2$ and with NaOH indicates that the pH of the filtrate became less after some precipitation had occurred than it was at the point where precipitation first began, in spite of the presence of additional increments of the base. Such behavior certainly appears anomalous; however, it has been observed before. Wendt and Clarke (15) ascribe it to the transitory nature of $CaHPO_4$ and to slowness in the establishment of a stable equilibrium. It could be due to differences in the degree of ionization of the acid phosphate.

It is of interest to notice in table 4 how the calcium in solution increased until the phosphorus began to precipitate, then decreased to a minimum with the phosphorus but rose again after all of the phosphorus had been withdrawn from solution.

Figures 1 and 2 show exceptionally well how valueless is a statement concerning the precipitation of phosphorus from solution by Ca^{++} ions or any other ions without consideration of the effect of anions present or of the pH of the system concerned.

The values recorded in table 6 show that the soil saturated with calcium acted somewhat as a sparingly soluble calcium salt from a weak acid in its precipitating effect upon phosphorus. Increasing amounts raised the pH of the filtrate and decreased the phosphorus in solution much as did increasing amounts of $CaCO_3$. It will be noted, however, that the curve for the soil is below that for $CaCO_3$, very probably because of adsorption by the large bulk of soil. Greater dilution appeared to have no pronounced additional effect upon phosphorus in solution or upon pH value but, as might be expected, it materially increased the amount of calcium in solution.

The data in table 7 and their graphical representation in figure 3 show very significant variations in the amounts of phosphorus in extracts from a soil saturated with calcium when the reaction was changed with HCl or different bases. When the pH of the system was sufficiently low, essentially all of the added phosphorus remained in solution; apparently, even that which would normally be adsorbed was held in solution. As the pH rose somewhat it appears that the Fe^{+++} ions, which had entered solution under the influence of the acid, precipitated some of the phosphorus between pH 2 and 3 where

$FePO_4$ has a minimum solubility; whereas, between pH 3 and 4, Al^{+++} ions, which also had been brought into solution by the acid, exerted their maximum effect upon the phosphorus and held the solubility low. At pH 5 the precipitating effect of Al^{+++} and Fe^{+++} ions was no longer in control, and Ca^{++} ions could not yet exert their maximum effect; hence, a maximum appeared in the solubility curve. As the pH was raised by different bases the curve descended again under the influence of the Ca^{++} ions.

The foregoing explanation is based upon the work of Gaarder (4), Teakle (14), Patten (11), and Line (7), and upon the results presented in the preceding series of experiments.

The data included in tables 2, 4, and 7 show that small quantities of CaO exert a much more pronounced effect upon pH and phosphorus solubility than do large quantities of $CaCO_3$. No phosphorus, detectable by the method used, occurred in solution in these experiments at a pH above 7.46.

In no case did NaOH completely precipitate the phosphorus, even though the pH of the filtrate was raised to 8. The slight solubility and the slight degree of ionization of calcium soil together with the effect of the dissimilar Na^{+} ions were probably the main cause of incomplete precipitation. The minimum amount of soluble phosphorus recovered following the application of NaOH was less than that found in the same series when $CaCO_3$ was used. This also indicates that the pH must be near 7.5 before Ca^{++} ions can exert their maximum precipitating effect upon phosphorus.

SUMMARY

In order to study the effect upon the solubility of phosphorus when the pH of a system was changed by different reagents, various compounds of calcium, or a soil saturated with calcium alone and in varying combination, were added to portions of a standardized solution of H_3PO_4.

Conclusions drawn from the results obtained may be summarized as follows:

Ca^{++} ions did not precipitate phosphorus from solution until the pH approached 5.5. Maximum precipitation or minimum solubility was not reached until the pH approached 7.5.

Large excesses of $CaCO_3$ failed to precipitate completely the phosphorus from solution, apparently because of its slight solubility.

Slight additions of CaO reduced the phosphorus in the filtrate to a minimum at an average pH of 7.36 and held it there at the higher pH values.

Large excesses of $CaCl_2$ precipitated no phosphorus from solution until the pH was raised by NaOH. Minimum solubility occurred at pH 7.36 and remained constant when the pH was extended above this value.

The soil saturated with calcium precipitated phosphorus much like a sparingly soluble calcium salt from a weak acid. The phosphorus solubility curve with this soil is similar to that with $CaCO_3$ but, probably because of adsorption, is displaced with respect to it.

When the pH of the calcium soil was varied with HCl, the added phosphorus failed, until a pH of almost 0 was reached, to remain completely in solution A minimum appeared in the solubility curve at pH 2.56; then a gradual rise occurred to define a maximum at approximately pH 5. The minimum at pH 2.56 may be ascribed to the removal of phosphorus by Fe^{+++} ions which had entered the solution under the influence of the acid; whereas the

maximum at pH 5 may be ascribed to the fact that the Fe^{+++} and Al^{+++} ions could no longer exert their maximum precipitating effect upon the phosphorus, and the maximum precipitating effect of the Ca^{++} ions had not been attained. Additions of NaOH, CaO, and $CaCO_3$, respectively, lowered the curve from the maximum at pH 5, but to different extents and by slightly different paths. CaO caused complete precipitation of phosphorus at pH 7.46 and held it there at the higher pH values. $CaCO_3$ did not raise the pH above neutrality and did not cause complete precipitation of the phosphorus. NaOH raised the pH to 8, but complete precipitation of the phosphorus did not occur, probably because of the slight solubility and low degree of ionization of the soil.

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ESTIMATION OF AVAILABLE PHOSPHATES IN SOILS BY CO_2 EXTRACTION

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An extensive literature has grown round the problem of estimating available phosphates in soils. From the classical method of Dyer (3) to the recent method of Truog (6), the object has always been to simulate natural conditions. It has been admitted by practically every worker that the ideal reagent for extracting available phosphates would be a solution of CO_2. The difficulty so far has been the lack of some simple technique which could be easily reproduced and by which the results could be duplicated. It is the object of this paper to describe the development of such a technique. No claim is made as to the value of the results in predicting the behavior of the soil in the field. As a matter of fact, opinion will always be divided on that point. The proposed method is, however, recommended for trial in laboratories where better facilities exist for such tests.

The mechanism by which plants extract phosphates from soils is little understood. The most probable conjecture is that the CO_2 given out by the roots helps in dissolving the otherwise insoluble phosphates present in the soil. Most of the extraction methods are based on this conjecture. A notable departure from this idea is the alkaline extraction method of Das (2), the practical application of which has been emphasized by the work of Hockensmith et el. (4). The great importance of standardizing a method in which water saturated with CO_2 constitutes the extraction medium, lies in the fact that its application would form one of the strongest arguments for or against the CO_2 theory of root absorption.

EXPERIMENTAL

In standardizing a method of this nature, the following factors had to be taken into consideration to ensure reproducibility of results: temperature, since the solubility of CO_2 in water depends on this; time of reaction; soil-water ratio; and influence of $CaCO_3$.

The method of extraction and estimation will be described first; and the influence of the various factors will be discussed later.

Method of extraction and estimation

Extraction was done in a pyrex U-tube of the type shown in figure 1. This enabled a continuous stream of CO_2 to be passed through the soil suspension

held in the larger limb. The suspension was thus constantly agitated with a saturated solution of CO_2. An ordinary cylinder of compressed CO_2 is very suitable for the purpose and lasts for a long time.

The soil suspension after extraction for 15 minutes is filtered, and phosphate is estimated by Deniges' method as described by Chapman (1). Four cubic centimeters of a reagent containing 2.5 per cent ammonium molybdate in 10 N H_2SO_4 was taken in a 100-cc. measuring flask and 25 cc. of the solution under test added to it. The volume was made about 99.5 cc., 6 drops of a 2.5 per cent solution of stannous chloride in HCl was added, and the volume was made up to 100 cc. The color developed after 15 minutes was compared with 10 cc. of a standard phosphate solution, a solution of potassium dihydrogen phosphate

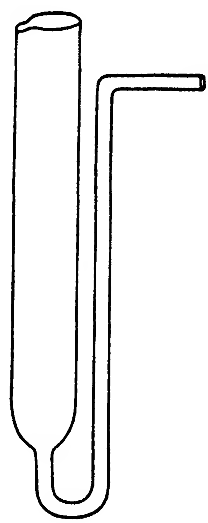

Fig. 1. Diagram of the U-Tube for the Extraction of Phosphates by Carbon Dioxide

containing 1 mgm. of P_2O_5 per 100 cc., in a colorimeter. The apparatus was standardized, and its limits of accuracy were determined with pure solutions before soils were tested.

Effect of temperature

Five soils were examined in this connection. CO_2 was passed for 15 minutes at different temperatures through 2 per cent soil suspensions. The results given in table 1 show that the temperature coefficient of the reaction is not high, though the effect is more marked in some soils than in others. Maximum value, however, is obtained at low temperature in all cases. Since it is easy to maintain a temperature about 5°C. with lumps of ice, this temperature is recommended. It will be noticed, however, that results at 15°C. are not so materially different, and if it is more convenient to use that temperature, there

should be no difficulty in duplicating the results. A temperature higher than 15°C. would not be desirable.

Effect of soil-water ratio

Effect of soil-water ratio was studied on P.C. 4 soil. The results given in table 2 show that the concentration of P_2O_5 in the CO_2 extract is practically independent of the soil-water ratio, except in 1 per cent suspension, where the value is slightly low. This result is very interesting and follows more logically from the conception of "availability" as the ability of the soil to supply P_2O_5. It would also appear that available P_2O_5 in a soil should not be expressed on the *weight of the soil* but in terms of the maximum concentration of the solution that can be obtained under a particular set of conditions. All results in this paper are recorded on this basis in milligrams of P_2O_5 per liter of the extracting solution. A 2 per cent suspension is recommended for routine work.

TABLE 1

Effect of temperature on the value of available P_2O_5 per liter obtained by CO_2 extraction

P.C. 2 SOIL		P.C. 4 SOIL		P.C. 10 SOIL		P.C. 16 SOIL		P.C. 17 SOIL	
Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5	Temperature	P_2O_5
°C.	mgm.	°C.	mgm.	°C.	mgm.	°C.	mgm.	°C.	mgm.
2.0	0.4	0 6	4 26	2 0	1 04	2.0	4 02	2 0	7.9
11.6	0.25	4 0	4 16	11 6	0 60	11 6	4 00	11 6	7.2
15.0	0 24	5.6	4 18	16 0	0 57	16.2	3 96	16 4	6 28
20.0	0.24	10 0	3 94	20 0	0 66	20 0	3 24	20.0	5.80
23.0	0.19	12 4	3.94	22 5	0 45	22 4	2 08	22 2	5.70
25 0	0.24	14 6	3 76	25 0	0 55	25 0	3 68	25 0	5 60
30.0	0 27	18.4	3 62	30.0	0 55	30.0	4 74	30 0	6.04
....	....	21 8	3 62	....	....	....	....	....	....
....	....	23.6	3 57	....	....	....	....	....	....
....	....	27 3	3.46	....	....	....	....	....	....
....	....	30 4	3 47	....	....	....	....	....	....

TABLE 2

Effect of soil-water ratio on available P_2O_5 in P.C. 4 Soil

SOIL PER 100 CC.	P_2O_5 PER LITER
gm.	mgm.
1	3 35
2	4.54
3	4.66
4	4.96
5	5 08
7.5	4.64
10	4 64

Effect of time of reaction

Effect of time of reaction was studied by passing CO_2 for varying lengths of time through a 2 per cent suspension of a soil rich in phosphates kept at 10–15°C. Results given in table 3 show that equilibrium is attained in about 10 minutes and that there is no appreciable increase when CO_2 is passed for a longer period. The effect of leaving the suspension for varying lengths of time before filtration after passing CO_2 for 15 minutes was also studied. One set was kept at 5°C. after CO_2 was passed, and the other set was kept at ordinary temperature (the temperature at the time of filtration is also recorded in this case). The results, given in table 4, show that the suspension after extraction can be kept for 2 hours at ordinary temperature without effect on the accuracy of the results. This shows that the P_2O_5 once extracted is not reprecipitated even when CO_2 is stopped. Ordinarily there would be no necessity of keeping the suspension so long after extraction, but the point was of interest as filtration takes some time, during which CO_2 may be lost. It is clear, however, that there is no likelihood of any error being introduced on that account.

TABLE 3

Effect of time of extraction on available P_2O_5

TIME	P_2O_5 PER LITER
minutes	*mgm.*
5	8.4
10	9.6
15	9.56
30	9.68
60	9 68
90	9 60
120	9.68

TABLE 4

Effect of keeping the suspension for different lengths of time after passing CO_2

TIME	TEMPERATURE WHEN FILTERED	P_2O_5 PER LITER
minutes	*°C.*	*mgm.*
0	3.5	9.08
10	10.2	8.64
10	3.5	8.84
15	11.8	9.48
15	3.5	8.80
30	15.0	9.48
30	3.5	8.48
60	20.0	9.12
60	3.5	8.48
90	22.1	8.92
90	3.5	8.40
120	24.3	8.80
120	3.5	8.70

Influence of $CaCO_3$

There is ample evidence in the literature to show that phosphates when added to calcareous soils are rendered unavailable. Thus Scarseth and Tidmore (5) found that yield with a high dressing of tricalcium phosphate was decreased about 60 per cent by the $CaCO_3$ when it was applied at planting, and

TABLE 5

Effect of $CaCO_3$, $CaSO_4$, or $CaCl_2$ on available P_2O_5

$CaCO_3$	P_2O_5 PER LITER	$CaSO_4$	P_2O_5 PER LITER	$CaCl_2$	P_2O_5 PER LITER
per cent	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
0	8 4	....	....	....	....
1	1.68	0.2	7.9	0.55	6 9
2	0.70	0 4	7.12	1.10	5 92
3	0 66	0 6	7 06	1 65	5.84
4	0.50	0 8	7 5	2.2	5.76
5	0.50	10 0	1.51	....	....
6	0.45	20.0	0 72	....	....
7	0 47	30 0	0 64	....	....
8	0 41	...	. ..	..	....
9	0.41	.. .	. ..	....	. ..
10	0.41	....	. ..	. ..	....

TABLE 6

Replicate determinations of available P_2O_5 in soils

SOIL NUMBER	P_2O_5 PER LITER							
	Rubbing				Without rubbing			
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	0 26	0 28	0 26	0 26	0.24	0 26	0 28	0 28
2	0 0	0 0	0 0	0 0	0.0	0.0	0 0	0 0
3	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
4	0 0	0 0	0 0	0 0	0 0	0 0	0.0	0 0
5	2.66	3 04	3.22	2 80	2.52	2 82	2 80	2 82
6	4 60	5.52	4 80	4 60	4 64	4 60	4 70	4.60
7	0 28	0 20	0 20	0 23	0 22	0 22	0.23	0 22
8	0.93	0.92	0 96	1 00	0.93	1 04	1.03	0.98
9	3.62	3 52	3.62	3 65	3 55	3 65	3 68	3.62
10	2.14	2 50	2 58	2.70	2 72	2 63	2 60	2.60
11	0.58	0.56	0 56	0.58	0 57	0 68	0 56	0 59
12	7 02	7 24	7 24	7 21	7 36	7 40	7 20	7 30
13	7 72	8.0	7 92	7 95	7 96	8 04	7 92	8 02
Dates...	6/2/34	7/2/34	10/2/34	15/2/34	6/2/34	7/2/34	10/2/34	15/2/34

about 97 per cent by the $CaCO_3$ when it was applied 180 and 365 days before planting. These authors put forward the view that calcium in the soil solution probably united with soluble phosphorous to form tri-calcium phosphate, thus lowering the availability of the phosphorous to plants and decreasing the yield.

The question of whether the method of finding available phosphates in soil should be independent of the amount of $CaCO_3$ contained in it is important, therefore. If $CaCO_3$ decreases the availability of phosphates as measured by crop yield, obviously the laboratory method must show a decrease. This is actually the case with the CO_2 extraction method, as will be seen from table 5, which records the values for available P_2O_5 with increasing amounts of $CaCO_3$. The effect of $CaSO_4$ and $CaCl_2$ was also studied, and the results are included in table 5.

It appears that all the Ca salts have a depressing effect on available phosphates, though the influence is most marked in the case of $CaCO_3$. The importance of applying phosphatic manures as top-dressing to calcareous soils thus becomes apparent.

Reproducibility of results

It is obvious that reproducibility of results is one of the first essentials of an analytical method. To test this point, 13 soils were selected at random.

TABLE 7

Comparison of Truog method and CO_2 extraction method

SOIL NUMBER	P_2O_5 PER LITER	
	Truog's method	CO_2 extraction method
	mgm.	*mgm.*
5	6.24	8.0
6	3.4	5.24
7	3.32	3 16
9	5.0	2.84
18	1.18	0.65
27	13.68	9.28
29	1.00	1.56
41	0.5	0.38

Available phosphate was determined eight times on different days. The experiment was divided into two series: in one, the soil was thoroughly rubbed with a rubber pestle before extraction, and in the second, no preliminary treatment was given. The results recorded in table 6 leave no doubt as to the reproducibility of results. It is also seen that rubbing with a rubber pestle has no effect.

Comparison with Truog method

The CO_2 extraction method was compared with the Truog method in which the extraction is done with a buffer mixture of H_2SO_4—K_2SO_4 of pH 3. The results, given in table 7, show a general similarity, which is evidently due to the fact that extraction is effected at approximately the same pH value in both cases.

SUMMARY

A simple method for determining available phosphates in soils by CO_2 extraction has been described.

The method has been shown to give reproducible results with a number of soils. It is free from all complicated details, and the results are unaffected by alterations in the soil-water ratio, the time of extraction, etc. The addition of $CaCO_3$ to the soil lowers the value for available phosphate as determined by this method.

The method shows a general agreement with the Truog method.

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ESTIMATING EXCHANGEABLE CALCIUM AND OTHER CATIONS IN SOILS

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I. THE SODIUM CARBONATE METHOD OF ESTIMATING EXCHANGEABLE Ca IN CALCAREOUS SOILS

Estimation of exchangeable Ca in calcareous soils has always presented peculiar difficulties on account of the solubility of calcium carbonate in the reagent used for replacement. Estimating Ca in the second liter of the leachate and thus allowing for the solubility effect (1) entail double the work. The use of alcoholic solutions brings in analytical difficulties besides the expense.

The writer has recently suggested an indirect method for such soils (5) which consists in leaching the soil with ammonium carbonate when all the bases are replaced by ammonia. The latter is determined, and from the total bases thus found, bases other than Ca which appear in the filtrate are subtracted; the difference is equivalent to exchangeable calcium.

Since ammonium carbonate can convert all the exchangeable Ca into carbonate; it was logical to suppose that sodium carbonate would behave in a similar manner. If Na_2CO_3 or K_2CO_3 could convert all the exchangeable Ca into carbonate, the decrease in the CO_2 concentration of a standard solution of Na or K carbonate would be equivalent to exchangeable Ca. The present paper deals with the application of this principle to the estimation of exchangeable Ca in calcareous soils.

Experimental

In order to standardize working conditions, it was considered necessary to perform some preliminary experiments. The results of these experiments, it was hoped, would lead to a clearer understanding of the nature of the reactions involved in the new method.

The success of the sodium carbonate method must obviously depend on the insolubility of calcium carbonate in sodium carbonate solution. In order to test this point 200-cc. portions of approximately 0.05 N and 0.1 N Na_2CO_3 solution were shaken with $CaCO_3$ in the presence of increasing amounts of NaCl. After the $CaCO_3$ was allowed to settle, 100 cc. of the clear supernatant liquid was pipetted off; excess of standard HCl was added; the liquid was boiled to expel CO_2 and back-titrated with standard alkali. The results

given in table 1 show that $CaCO_3$ is practically insoluble in Na_2CO_3 solution even in the presence of NaCl.

In order to determine whether the presence of NaCl is necessary for the complete precipitation of exchangeable Ca in the soil, 10-gm. portions of a calcareous soil (from Akola) were shaken for 2 hours with 200 cc. of 0.05 *N* Na_2CO_3 containing increasing amounts of NaCl. The suspension was then filtered and 100 cc. of the filtrate titrated with standard acid using first phenolphthalein and then methyl orange as indicator, the decrease in the concentration of CO_3 ion being taken as equivalent to exchangeable Ca. The results given in table 2 show that the value is slightly low in the absence of NaCl, but increasing amounts of the latter have very little effect.

The effect of time of shaking and contact with Na_2CO_3 solution was next studied on the same soil (from Akola). Ten-gram portions of the soil were shaken with 100 cc. of 0.1 *N* Na_2CO_3 in 0.1 *N* NaCl for varying intervals of time. Exchangeable Ca was determined by noting the decrease in the CO_3 concentration as before. The results given in table 3 show that equilibrium is established in 2 hours.

TABLE 1

Insolubility of $CaCO_3$ *in* Na_2CO_3 *plus* NaCl *solutions*

CONCENTRATION OF NaCl	TOTAL ALKALINITY M.E. PER 100 CC.	
	1	2
0	5.49	10.97
0.05 *N*	5.51	10.95
0.1 *N*	5 50	11.00
0.2 *N*	5.46	10.91
0.5 *N*	5.44	10.76
1.0 *N*	5.36	10.88
Without $CaCO_3$	5.46	10.87

Detailed description of the proposed method

After several trials the following technique was found to be most suitable, though strict adherence to detail is not at all necessary as long as we bear in mind that the fundamental reaction involved is the precipitation of a Ca salt as $CaCO_3$, a reaction already well known in analytical chemistry:

Ten to twenty grams of soil is shaken for 2 hours with 200 cc. of 0.05 *N* Na_2CO_3 in *N* NaCl (K_2CO_3 and KCl serve equally well). The suspension is then filtered through a fluted filter, and 50 cc. of the filtrate is titrated as follows: Phenolphthalein indicator is added and standard HCl run in till the solution is colorless. The acid used is equivalent to half the carbonates in solution. A few drops of brom thymol blue indicator are then added, excess of acid is run in, and the solution is back titrated with standard alkali after boiling. The total quantity of acid added (including the phenolphthalein

titer) minus the alkali used for back titration gives the total alkalinity of the solution, i.e., the carbonate and bicarbonate. Bicarbonate is obtained by subtracting from this value twice the value for phenolphthalein titer.

The value of bicarbonate thus obtained is only approximate and is used as a guide to more accurate determination which is carried out as follows: To 100 cc. of the filtrate are added standard $BaCl_2$ and NaOH in quantities little more than the total alkalinity and bicarbonate respectively. The solution is then titrated with 0.1 *N* oxalic acid using thymolphthalein as indicator. The decrease in the concentration of NaOH added is equivalent to the bicarbonate in solution. Phenolphthalein can be used in place of thymolphthalein, but the latter gives a much sharper end point.

Since the same equivalent of bicarbonate contains twice as much CO_2 as does the carbonate, the total CO_2 in a mixture of carbonate and bicarbonate is obtained by adding the bicarbonate equivalent to total alkalinity.

TABLE 2

Exchangeable Ca *in a calcareous soil determined by* Na_2CO_3 *with and without* NaCl

CONCENTRATION OF NaCl	EXCHANGEABLE Ca, M E PER 100 CC.
0	44.4
0.05 *N*	48 4
0.10 *N*	48.8
0.2 *N*	50 4
0.5 *N*	49.6
1.0 *N*	49.2

TABLE 3

Effect of time of shaking on the value of exchangeable Ca

TIME OF SHAKING	EXCHANGEABLE Ca, M.E. PER 100 CC.
hours	
2	49.8
5	49.0
8	48.8
24	49.6
96	49.8

It might be pointed out that exchangeable Mg in soils is not precipitated along with Ca in this method but is brought into solution as carbonate. This obviously makes no change in the concentration of total CO_3 ions and therefore does not interfere in the Ca estimations. Incidentally, exchangeable Mg can be estimated in an aliquot of the filtrate. This point will be discussed more fully in a later publication dealing with the methods of estimating exchangeable Mg in soils.

In order to see how the Na_2CO_3 method compares with other methods, 14 soils were examined. The results given in table 4 show that there is good agreement among the different methods.

The application of well-known analytical principles to the estimation of exchangeable Ca in soils has resulted in the perfection of the ammonium oxalate method described in a previous publication (6) and the present sodium carbonate method. Apart from their utility as rapid methods of analysis, they are of great importance in so far as they support the chemical theory of soil colloids (3). The success of these methods leads one to the irrefutable conclusion that exchangeable Ca exists in chemical combination with the clay

complex, and the compound may for the present be regarded as calcium clayate, i.e., the calcium salt of clayic acid (4).

The fact that sodium carbonate in soils leads to the complete precipitation of exchangeable Ca throws an interesting side light on the cause of barrenness in alkali soils. It is not improbable that plants are unable to grow in such soils on account of calcium deficiency, no doubt brought about by the sodium carbonate which is invariably present in such soils.

It is interesting to recall in this connection the following conclusion of Joshi and Puri reached in their study on the influence of exchangeable ions in soil colloids on bacterial activity and plant growth (2): "There is no evidence

TABLE 4

Exchangeable Ca *in calcareous soils as determined by various methods*

SOIL NUMBER P. C.	pH VALUE	$CaCO_3$	EXCHANGEABLE Ca, M.E. PER 100 CC.		
			KCl method	$(NH_4)_2CO_3$ method	Na_2CO_3 method
		per cent			
1	8.41	36.05	5.1	3.95	5.3
2	8.21	4.87	49.9	52.35	55.7
4	8.55	0.82	6 25	7.05	5.4
5	8.77	1.64	6.00	6.10	5.3
7	9.58	5.62	0	1.40	1.9
8	8.41	0.58	16 7	16.90	18.3
10	8.71	1.02	19 25	19.16	23 5
11	8 77	2 32	22.25	21 45	25.9
13	8 53	1.85	49.2	53.8	55.3
16	8.74	2 24	3.5	4 10	2 9
17	8.20	0 67	7.65	7.80	7 8
19	8.40	1.17	16 80	19 50	18.1
21	8.25	3.31	8 80	10 45	6 1
24	8.59	2 02	4.40	6.35	1.0

to show that exchangeable sodium ions are directly toxic to plants. Their toxicity is most probably due to the adverse physical conditions set up by the exchangeable sodium." The statement may now be modified so that the "adverse condition" referred to therein may not be merely "physical" but most likely physico-chemical.

II. SINGLE-TREATMENT METHODS OF ESTIMATING EXCHANGEABLE Ca AND OTHER BASES IN SOILS CONTAINING CALCIUM CARBONATE OR GYPSUM

The potassium oxalate-acetate-carbonate method

The development of the potassium oxalate-acetate-carbonate method was based on the fact that soluble oxalates react with $CaCO_3$ only in a medium in which the latter is soluble and consequently the greater the solubility of $CaCO_3$, the greater is the amount that will react with oxalate in a given time. Since

$CaCO_3$ is practically insoluble in solutions of alkali carbonates, various mixtures of alkali carbonates and their corresponding chlorides and acetates were tried. A mixture of potassium chloride or acetate and K_2CO_3 appeared to be most suitable for the purpose. The effect of increasing amounts of K_2CO_3 in normal potassium chloride or acetate on the reaction between potassium oxalate and $CaCO_3$ as well as the precipitation of exchangeable Ca in the case of a black cotton soil (Akola) is shown in table 5.

One hundred cubic centimeters of solutions of 0.1*N* K oxalate in *N* KCl or *N* K acetate with increasing amounts of K_2CO_3 were shaken with 2 gm. of $CaCO_3$ in one series and with 10 gm. of soil in the other. After being shaken intermittently by hand for half an hour, the mixture was filtered through a dry fluted filter. Fifty cubic centimeters of the filtrate was titrated

TABLE 5

Removal of oxalate ion by $CaCO_3$ and Akola soil in the presence of increasing amounts of K_2CO_3

0.1 *N* K_2CO_3 in 100 cc. solution	Oxalate ion removed (amount of 0.1 *N* solution)			
	N KCl		*N* K acetate	
	$CaCO_3$	Soil	$CaCO_3$	Soil
cc.	*cc.*	*cc.*	*cc.*	*cc.*
0	....	....	5.8	59.3
5	5.6	59.0	2.9	56.7
10	1.8	55 4	1.3	52.4
15	0.6	52 6	0.6	52 4
20	0.4	50 8	0.6	51.4
25	0.2	49.2	0 5	50.0
30	0 4	49.0	0.6	49.4
40	0.25	46.6	...	....
50	0.2	45.6	. .	....
75	0.1	42 4	.	....
100	0	39.8	...	....

with standard $KMnO_4$ and decrease in the concentration of oxalate noted. This decrease is equivalent to the $CaCO_3$ that reacted with K oxalate in the one case and with the exchangeable Ca in the other. It is seen that above a certain concentration of K_2CO_3 there is practically no reaction between K oxalate and $CaCO_3$; but exchangeable Ca, as determined by the disappearance of the oxalate ion, continues to decrease. What actually happens is that when the concentration of K_2CO_3 is small some K oxalate reacts with $CaCO_3$ in the soil, giving too high a value for exchangeable Ca. At the point where the reaction between K oxalate and $CaCO_3$ ceases, the decrease in oxalate ion is equivalent to exchangeable Ca. When the amount of K_2CO_3 is increased still further, there is a competition between K oxalate and carbonate to precipitate Ca as oxalate or carbonate. Since increasing amounts of K_2CO_3 would lead to the formation of increasing amounts of $CaCO_3$, the value for

exchangeable Ca decreases continuously. The logical conclusion is that the concentration of K_2CO_3 should be kept within certain limits so as to render $CaCO_3$ insoluble but not to induce the precipitation of exchangeable Ca as carbonate.

Other experiments showed that K acetate is superior to KCl in the oxalate-carbonate mixture, as the latter gives slightly low values in the case of non-calcareous soils. The use of K oxalate-acetate-carbonate mixture ensures one uniform procedure for calcareous as well as carbonate-free soils.

The most suitable medium having been found for the precipitation of Ca oxalate in the presence of $CaCO_3$, an experiment was performed to determine whether the amount of $CaCO_3$ had any effect on its reaction with K oxalate. It was found that when the amount of $CaCO_3$ was increased from 1 to 10 gm., the portion that reacted with K oxalate remained practically constant. It was concluded, therefore, that the percentage of $CaCO_3$ in a soil will have no influence on the determination of its exchangeable Ca by this method.

The effect of temperature on the rate of reaction was next studied. It was found that higher temperatures slightly favored carbonate formation, that a temperature below 20°C. was most favorable to the exclusive formation of Ca oxalate, and that a half hour's shaking by the hand was quite sufficient for the completion of the reaction. In tropical countries like India where the temperature in summer is high, an ordinary galvanized iron tank containing water cooled to 15°C. with ice may be used. No temperature control is required; it is only necessary to avoid too high a temperature.

Summary description of the method. Ten grams of soil is shaken in a stoppered bottle by the hand or in an end over end shaker for about half an hour (in the case of heavy soils the shaking is continued for 1 hour) with 100 cc. of a solution N with respect to K acetate, 0.1 N with respect to K oxalate, and 0.015 N with respect to K_2CO_3, filtered, and 50 cc. of the filtrate titrated with 0.1 N $KMnO_4$. The total decrease in the concentration of oxalate ion is equivalent to exchangeable Ca in the given weight of the soil. If the room temperature is high the suspension should be cooled to 15°C.

The ammonium oxalate-acetate-carbonate method

It was found in the preliminary experiments that when ammonium salts were substituted for potassium salts $CaCO_3$ reacted with the oxalate ion to a large extent. It appeared, however, that, though the analogy broke down when equivalent concentrations were used, there might be a different proportion of the oxalate-acetate-carbonate mixture in the case of ammonium salts in which $CaCO_3$ might be insoluble. The additional advantage of such a mixture would be that ammonium salts could be removed, leaving behind all the other exchangeable bases as carbonates, and therefore the method could be used for determining other cations.

In the application of the ammonium oxalate method to calcareous soils three important factors were determined: the proportion of ammonium car-

bonate that would render $CaCO_3$ insoluble but would not precipitate exchangeable Ca as carbonate—0.25 N; the optimum temperature at which the reaction should be allowed to take place—about 10°C.; and the time of shaking the soil with the reaction mixture—half an hour.

Summary description of the method. Twenty grams of soil is added to 200 cc. of a solution 0.1 N with respect to ammonium oxalate, 0.5 N with respect to ammonium acetate, and 0.25 N with respect to ammonium carbonate, cooled to 10°C.

The Ca is determined as in the K oxalate-acetate-carbonate method. For the other cations 100 cc. of the filtrate is evaporated to dryness and gently ignited, care being taken to avoid spurting. The residue, which consists of the carbonates of exchangeable Na, K, and Mg, is taken up with the least amount of boiled water and filtered, followed by one or two leachings. The combined filtrate consists of exchangeable Na and K; whereas the residue on the filter paper is exchangeable Mg as carbonate. Both are titrated separately with standard acid, directly in the case of Na and K, residually in the case of Mg.

Soils rich in organic matter sometimes yield a filtrate which is highly colored and therefore cannot be titrated directly with permanganate. For such soils the following modification is suggested: Fifty cubic centimeters of the filtrate is acidified with acetic acid, and the oxalic acid is precipitated by adding excess $CaCl_2$ to the hot solution. Since the maximum amount of oxalic acid present is known, a great excess can be easily avoided. The precipitate of calcium oxalate is filtered, washed, and ignited to convert it into carbonate or oxide, which is then titrated residually after excess standard acid has been added. This titer is equivalent to the oxalate present in the solution, from which the decrease in the concentration of oxalate ion is computed. Other bases are, of course, determined as usual by igniting an aliquot and titrating the residue.

Comparison with other methods

The following methods were tried:

(A) *NaCl method.*—Ten grams of soil is leached with 1000 cc., of 0.2 N NaCl in 100-cc. lots, followed by another 1000-cc. leaching in the same way. Ca is determined in the two leachates separately, and the difference between the two is taken as equivalent to exchangeable Ca.

(B) *KCl method.* Exactly like (A) but 0.2 N KCl is used instead of NaCl.

(C) *Ammonium acetate method.* Ten grams of soil is leached with 1 l. of N ammonium acetate in 100-cc. lots; Ca is determined in an aliquot of the leachate. This method is used for carbonate-free soils only.

(D) *NH_4Cl method.* Ten grams of soil is leached with 1 l. of N NH_4Cl in 100-cc. lots; Ca is determined in the leachate. This method is used for carbonate-free soils only.

(E) *$(NH_4)_2CO_3$ method.* Ten grams of soil is leached with 500 cc., of N

$(NH_4)_2CO_3$. Total ammonia taken up by the soil minus the exchangeable Na, K, and Mg in the leachate is taken as equivalent to exchangeable Ca (5).

(*F*) *Na_2CO_3-NaCl method.* Ten grams of soil is shaken for 2 hours with 200 cc. of 0.1 *N* Na_2CO_3 solution in *N* NaCl. An aliquot of the filtrate is

TABLE 6

Exchangeable Ca in calcarious soils by various methods

SOIL NUMBER	$CaCO_3$	pH	CLAY	EXCHANGEABLE Ca, per 100 GM. SOIL								Na PLUS K	Mg
				(A)	(B)	(E)	(F)	(G)	(H)	(I)	(J)		
	per cent		*per cent*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1	36.05	8.41	11.3	5.4	5.1	3.95	5.3	4.8	7.6	4.1	8.4	0.4	1.7
2	4.87	8.21	59.3	47.9	49.9	52.35	55.7	49.6	46.2	48.0	48.0	2.3	10.0
4	0.82	8.55	15.2	9.15	6.25	7.05	5.4	8.4	9.0	7.4	8.4	0.4	0.6
5	1.64	8.77	12.3	7.00	6.00	6.10	5.3	8.4	9.1	6.6	8.6	0.2	1.0
7	5.62	9.58	21.8	3.00	0	1.4	1.9	3.4	3.9	2.2	2.0	9.2	5.8
8	0.58	8.41	25.2	18.35	16.7	16.9	18.3	19.8	21.2	19.0	19.0	1.0	2.0
10	1.02	8.71	35.6	20.75	19.25	19.16	23.5	23.2	25.5	22.8	22.0	1.5	3.3
11	2.32	8.77	32.8	23.1	22.25	21.45	25.9	27.6	28.1	26.0	28.4	0.8	4.6
13	1.85	8.53	58.9	48.3	49.2	53.8	55.3	54.8	55.6	50.6	51.0	0.4	5.5
16	2.24	8.74	7.3	3.6	3.5	4.1	2.9	3.6	5.8	4.4	5.8	0.25	2.9
17	0.67	8.20	14.2	9.0	7.65	7.8	7.8	10.4	8.6	7.2	....	...	...
19	1.17	8.40	42.4	15.4	16.8	19.5	18.1	21.0	19.0	17.2	18.0	0.5	3.0
21	3.31	8.25	13.5	7.05	8.8	10.45	6.1	11.4	12.0	9.0	....	..	...

TABLE 7

Exchangeable Ca in carbonate-free soils by various methods

SOIL NUMBER	pH	CLAY	EXCHANGEABLE Ca, PER 100 GM. SOIL							Na PLUS K	Mg
			(C)	(D)	(F)	(H)	(I)	(J)	(K)		
		per cent	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
3	7.64	62.3	53.8	50.8	53.2	55.8	53.4	51.4	59.6	1.1	12.4
6	5.29	28.4	5.1	4.9	0.6	6.3	3.8	4.2	5.4	0.3	1.5
9	5.76	21.6	3.8	4.45	0.6	4.2	1.3	2.2	4.6	0.9	0.8
12	5.84	3.8	3.6	3.95	0	4.4	0	2.2	3.2	0.7	1.6
14	5.37	22.3	2.8	3.75	5.7	5.6	6.0	5.4	5.0	0.15	0.65
15	7.71	21.9	13.0	13.9	12.0	12.0	8.6	8.6	12.0	0.1	0.9
18	5.79	22.2	4.1	4.3	4.5	4.0	2.2	...	3.6	...	...
20	5.64	6.5	3.1	4.8	4.5	2.3	...	0.2	2.2	0.15	1.2
22	6.85	15.2	7.85	8.1	8.2	8.5	...	...	8.5	...	...
23	7.41	11.3	8.2	9.05	5.6	8.4	6.5	...	9.0	...	...

titrated with standard acid using phenolphthalein first and then methyl orange as indicator. The decrease in the concentration of CO_3 ion is taken as equivalent to exchangeable Ca (see Part I).

(*G*) *K_2CO_3-KCl method.* Exactly like (*F*) but K salts are used instead of Na salts.

(*H*) *K oxalate-acetate-carbonate method.* Method exactly as described in this paper.

(*I*) *K oxalate-chloride-carbonate method.* Exactly like (*H*) except that KCl is used in place of K acetate.

(*J*) *Ammonium oxalate-acetate-carbonate method.* Exactly as described in this paper.

(*K*) *Ammonium oxalate-acetate method.* Exactly like (*J*) but without $(NH_4)_2CO_3$. This method is used for carbonate-free soils only.

The results of this comparison are given in tables 6 and 7. Exchangeable Na + K, and Mg in the soils were determined by the ammonium oxalate-acetate-carbonate method. The various methods show a good agreement when the nature of a material like soil and the inaccuracies of the leaching methods are considered.

The general agreement among the various methods also disposes of the question of the fate of oxalate in soils. It is quite clear that we need not have recourse to absorption phenomenon to explain its disappearance. One or two highly acid soils were encountered which yielded with the oxalate-acetate-carbonate mixture an extract which was capable of being oxidized by permanganate solution, thus leading to low values for exchangeable Ca. The amount thus brought out was never more than 1 to 2 m.e. per 100 gm. of soil, however, and could always be allowed for by running a blank.

Application of the single-treatment methods to soils containing gypsum

The methods discussed in the foregoing, obviously, are not applicable to soils containing gypsum, which must be removed or rendered insoluble before they are applied. A technique was developed by which all the $CaSO_4$ is converted into $CaCO_3$. Briefly, the method consists in shaking the soil suspension with excess $BaCO_3$ when the following reaction takes place:

$$CaSO_4 + BaCO_3 \rightarrow CaCO_3 + BaSO_4$$

The excess $BaCO_3$, as well as the products of reaction, is insoluble in the medium in which the final precipitation of exchangeable Ca is effected.

For standardizing experimental conditions, gypsum was added in known amounts to a black cotton soil from Akola, since soils containing varying quantities of gypsum were not available. The effectiveness of the method of removing $CaSO_4$ was judged by the nearness of the value of exchangeable Ca in the treated soil to its true value in untreated Akola soil as previously determined by various methods.

Speed of reaction between $CaSO_3$ and $BaCO_3$. On account of the limited solubility of both $CaSO_4$ and $BaCO_3$, it was necessary to study the time required for the complete precipitation of $CaSO_4$. To 10-gm. portions of Akola soil were added 0.5 gm. of $CaSO_4$ and 1 gm. of $BaCO_3$. The mixture was shaken with 50 cc. of water for varying lengths of time, and exchangeable Ca was determined as described in this paper. Controls were run with no soil.

It was assumed that when the reaction between $CaSO_4$ and $BaCO_3$ was complete, the soil would give the original value for exchangeable Ca. The results given in table 8 show that in the case of soil and gypsum 2 hours' shaking is quite sufficient to bring about the complete precipitation of $CaSO_4$ so that practically the same value for exchangeable Ca is obtained as without gypsum. It is, of course, possible that in natural soils gypsum may not be in such a soluble state and longer shaking may be required, but this could be easily ascertained by individual workers for the soils they might be dealing with. In any case, overnight shaking is recommended in the standard technique to insure completion of the reaction.

It might be noted that when dilute H_2SO_4 is added to the filtrate prior to titration with $KMnO_4$, a white precipitate of $BaSO_4$ is noticed. This is due

TABLE 8

Speed of reaction between $CaSO_4$ and $BaCO_3$

MIXTURE	TIME OF SHAKING	OXALATE REMOVED
	hrs.	*m.e.*
$CaSO_4$ + $BaCO_3$	0	5.18
	0.5	0.89
	1	0.49
	2	0.26
$CaSO_4$ + $BaCO_3$ + soil	2	5.23
	5	5.30
	10	5.40
	20	5.38
Soil alone	..	5.24
$CaSO_4$ alone	..	5.45
$BaCO_3$ alone	..	0

to the presence of some barium oxalate in the solution and does not interfere with the titrations or the accuracy of the method.

Next, the effect of increasing amounts of $CaSO_4$ in Akola soil on the estimation of exchangeable Ca in it was tried: 0.5 to 2 gm. of $CaSO_4$ were added to 10 gm. of soil. This corresponded with 5 to 20 per cent of gypsum. The amount of $BaCO_3$ was increased proportionately and was kept at double the amount of $CaSO_4$ per 10 gm. of soil. The results given in table 9 show that the amount of exchangeable Ca is practically unaffected by the presence of $CaSO_4$, provided the soil is first shaken with $BaCO_3$.

In order to determine whether the principle just enunciated was applicable to other types of soils, a number of calcareous soils were examined for their exchangeable Ca, before and after the addition of 5 per cent gypsum and shaking with $BaCO_3$. The results given in table 10 show that in most cases there is a slight increase in the exchangeable Ca after the addition of gypsum.

This is evidently because the soils that were not in equilibrium with $CaSO_4$ took some more calcium in the exchangeable form, resulting in a higher value. It is important to note, however, that this slight increase (the highest being 0.6 m.e.) could not account for the whole of the $CaSO_4$ (5.8 m.e.) added to the soil.

Detailed description of the proposed method. Ten grams of soil is shaken overnight with 50 cc. of water and 1 gm. of $BaCO_3$ in a stoppered bottle, cooled to 15°C., and 50 cc. of the potassium or ammonium oxalate-acetate-

TABLE 9

Exchangeable Ca in Akola soil with increasing amounts of $CaSO_4$ in 10 gm. of soil

GYPSUM ADDED	$BaCO_3$ ADDED	EXCHANGEABLE Ca
gm.	*gm.*	*m.e.*
0	0	5.62
0.5	1	5 64
1.0	2	5.74
1.5	3	5.82
2.0	4	5.90

TABLE 10

Exchangeable Ca in soils with and without gypsum

SOIL NUMBER	CLAY	pH	EXCHANGEABLE Ca PER 100 GM. SOIL	
			With gypsum	Without gypsum
	per cent		*m.e.*	*m.e.*
1	11.3	8.41	10.4	9.6
4	15.2	8.55	15.8	9.0
5	12.3	8.77	14.6	9.1
7	21.8	9.58	3.0	3.9
8	25.2	8.41	25.8	21.2
10	35.6	8.71	31.8	25.5
11	32.8	8.77	33.0	30.0
13	58.9	8.53	56.6	55.6
16	7.3	8.74	11.4	5.8
17	14.2	8.20	14 4	8.6
21	13.5	8.25	14.3	12.1

carbonate mixture is added. The procedure followed is the same as for the K oxalate-acetate-carbonate method.

If the soil contains sulfates of alkali metals besides $CaSO_4$, these must be removed by leaching with alcohol (rectified spirit), before the soil is shaken with $BaCO_3$. The reason for this precaution is that alkali sulfates can react with $BaCO_3$ to give $BaSO_4$ and alkali carbonates, and the latter can react with exchangeable Ca and remove it as carbonate (6); therefore, at the end of the first reaction some of the exchangeable Ca might be precipitated along with the

$CaSO_4$. Leaching with alcohol leaves the soil in a friable state on drying, and it can be detached easily from the filter paper for the determination of exchangeable Ca.

The influence of alkali sulfates will be readily understood from the following experiment in which 10-gm. portions of Akola soil were shaken with 0.5 gm. of $CaSO_4$ and 1 gm. of $BaCO_3$ in the presence of increasing amounts of sodium sulfate. The results given in table 11 show that the amount of exchangeable Ca decreases as the sodium sulfate is increased in the soil. The decrease in

TABLE 11

Effect of Na_2SO_4 on exchangeable Ca in Akola soil in the presence of 5 per cent gypsum

Na_2SO_4 PER 100 GM. SOIL	EXCHANGEABLE Ca PER 100 GM. SOIL AFTER $BaCO_3$ TREATMENT
m.e.	*m.e.*
0	52.4
10	44 4
20	36.4
30	31.0
40	29.2
50	27.0

TABLE 12

Various forms of Ca in Sind soils

SOIL NUMBER	SOLUBLE SALTS	Ca PER 100 GM. SOIL IN VARIOUS FORMS		
		Exchangeable	$CaSO_4$	$CaCl_2$
	per cent			
1	18.2	3.2	5.2	28.4
3	15.76	5.2	2.4	29 8
4	29.18	4.6	30.6	19.6
7	23.02	4.8	23.6	25.8
8	10.74	0	15.6	3.6
9	2 24	7.0	9.4	7.0
10	57.4	2.2	32.0	7.0
12	13.28	6.4	2.8	30.0

exchangeable Ca, however, is not proportional to the Na_2SO_4 present; in fact, after the first 3 m.e., the effect is very much reduced.

As an example of the practical application of the results of this investigation, the analyses are recorded of certain soils from Sind which contained $CaCl_2$, $CaSO_4$, and Na_2SO_4. Incidently the technique developed herein enables us to differentiate between the various forms of Ca. The following determinations were made: (A). The soil was leached with 200 cc. of alcohol, and the filtrate was examined for $CaCl_2$ and alkali sulfates. (B). The alcohol-leached soil was shaken with 50 cc. of water and 1 gm. of $BaCO_3$ and then

examined for exchangeable Ca. (C). The alcohol-leached soil was examined for exchangeable Ca without being shaken with $BaCO_3$. (A) gave $CaCl_2$, (C)—(B) gave $CaSO_4$, and (B) gave exchangeable Ca.

The results given in table 12 bring out clearly the characteristic difference in the soils. It will be seen that a number of soils, although containing a large amount of water-soluble Ca, have very little exchangeable Ca. This is, of course, due to a very large excess of sodium salts.

SUMMARY

A new method of estimating exchangeable Ca in calcareous soils has been described. It consists in shaking the soil with 0.05 N Na_2CO_3 in N NaCl. The decrease in the concentration of CO_2 ions is equivalent to exchangeable Ca in the soil.

The importance of the method in supporting the chemical theory of soil colloids and its bearing on the cause of barrenness in alkali soils are pointed out.

Exchangeable Ca in calcareous soils can also be determined by shaking a known weight of the soil with a definite mixture of potassium or ammonium oxalate-acetate-carbonate. In this mixture $CaCO_3$ is insoluble, and the decrease in the concentration of oxalate ion is equivalent to exchangeable Ca.

Soils containing gypsum require a preliminary treatment with excess $BaCO_3$ when the $CaSO_4$ is converted into $BaSO_4$ and $CaCO_3$, and thus rendered insoluble.

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ROOT NODULE BACTERIA OF SOME TROPICAL LEGUMINOUS PLANTS: I. CROSS-INOCULATION STUDIES WITH *VIGNA SINENSIS* L.[1]

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The contributions of Lewis and Nicholson (9), Maassen and Müller (11, 12), Simon (18), Garman and Didlake (5), Burrill and Hansen (2), and others have established the classification of leguminous plants into cross-inoculation groups with respect to the root nodule-forming bacteria. In the last two decades so much information has accumulated concerning this relationship between the host plant and its nitrogen-fixing symbiont that at present about 20 recognized groups exist.

Of those plants which have been placed in cross-inoculation groups the majority are forage or cover crops common to temperate regions, although many have their origin in tropical and subtropical areas. Relatively few large shrubs and trees have been investigated. The present study has had a two-fold objective: (*a*) a survey of the nodule formation on the root systems of some of the more readily accessible leguminous plants growing in the Territory of Hawaii, and (*b*) the isolation of Rhizobia from the nodules of tropical species to be used as inocula in plant tests. The data reported, accumulated over a period of 5 years, concern various leguminous vines, shrubs, and trees found only in the botanical gardens and arboreta on the island of Oahu, and other leguminous plants more or less common throughout the Territory.

The Territory of Hawaii has an extensive leguminous flora. Although Rock (15) listed only 70 genera and 200 species of leguminous plants for the Territory of Hawaii in 1918, the number of species, because of the careful and extensive introductions made by various societies and experiment stations, has probably doubled in the past 18 years. For most of these introductions credit is due the Experiment Station of the Hawaiian Sugar Planters' Association. Practically all of the leguminous plants concerned in this study were introduced into Hawaii from other tropical areas, since only 8 of the 22 indigenous species listed by Rock (15) are mentioned.

EXPERIMENTAL

The scientific name of each plant studied was regarded of primary importance, the common name as secondary. In those instances where the authors

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were in doubt as to the true scientific name of the plant, plant parts were brought into the laboratory and taxonomic keys consulted. Frequently taxonomic experts accompanied the authors on the various field trips and classified the plants *in situ*. Ultimately the scientific names of all the plants herein reported were checked and verified by competent taxonomists of island flora.[2]

Various treatises on tropical flora, in addition to *Index Kewensis*, have constituted the background for the plant classifications. Rock's monographs on the *Leguminous Plants of Hawaii* and the *Indigenous Trees of the Hawaiian Islands* were especially valuable. For the naming of the plants more recently introduced, the contributions of Merrill (13), Ridley (14), Britton and Wilson (1), and others were consulted.

DISTRIBUTION AND DESCRIPTION OF NODULES

Root systems of all the plants listed in table 1 were examined during the growth of the plants in forest or field soil. Although, because of the variables naturally encountered, no attempts were made to determine quantitatively the number of nodules per plant, an effort was made to ascertain the relative extent of nodulation. In the cases of the trees this aim was accomplished by a careful removal of the soil from a large portion of the root mass. On a few occasions when this procedure was thought inadequate, a more extensive examination was made by removing practically the entire root system of the species from the soil. Root systems of the vines, herbs, and many of the shrubs such as the species of Indigofera, Crotalaria, and Alysicarpus, were, of course, removed from the soil without difficulty. When the soil was too rocky, hard, or sticky to permit a satisfactory examination of the root systems, seeds were collected and subsequently planted in various lots of island soil, and the resulting plants were examined during periods of growth.

There seemed to be no correlation between the age or size of any of the plants and the extent of nodulation. This was of particular interest, since the ages of the trees examined varied from 1 to 20 years and their heights from 5 to 40 feet. Considerable variations occurred in the sizes, numbers, and distribution of nodules on any one species of the trees examined. As a rule, the young nodules were round, white, and smooth-surfaced, whereas the older forms assumed variously branched shapes, were commonly black or dark brown and rough-surfaced, and tended to be more or less of a hard "corky" or "woody" texture. Nodules on the species of Erythrina and Lonchocarpus were usually more or less centrally located on the upper root systems immediately around

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TABLE 1

Additions to the cowpea group on the basis of reciprocal cross-inoculation with Vigna sinensis _L._

Sub-family MIMOSOIDEAE

Enterolobium cyclocarpum Griseb.
Samanea saman (Jacq.) Merrill
*_Albizzia lebbek_ (L.) Benth.
Albizzia retusa Benth.
Albizzia chinensis (Osbeck) Merrill
Albizzia saponaria (Lour.) Blume
Albizzia moluccana Miq.
Albizzia odoratissima Benth.
Albizzia acle (Blanco) Merrill
Albizzia lebbekoides (DC.) Benth.
Albizzia procera (Roxb.) Benth.
Albizzia katangensis Wildem.
*_Acacia koa_ A. Gray
Acacia arabica Willd.
Acacia koaia Hillebr.
Acacia confusa Merrill
Acacia horrida (L.) Willd.
Acacia catechu Willd.
Acacia scorpioides A. Cheval
Acacia macrantha H. and B.
Acacia spadicigera Cham. and Schlecht
Acacia robusta Burch.
Acacia richii A. Gray
Acacia auriculaeformis A. Cunn.
Acacia baileyana F. v. M.
Acacia decurrens Willd.
*_Acacia farnesiana_ (L.) Willd.
Acacia glaucescens Willd.
Acacia verticillata Willd.
Inga edulis Mart.
Inga laurina (Sw.) Willd.
Parkia africana R. Br.
*_Parkia biglandulosa_ Wight and Arn.
Parkia javanica (Lam.) Merrill

Sub-family CAESALPINIOIDEAE

*_Cassia mimosoides_ L.
Cassia pilifera Vog.
Hymenaea courbaril L.

Sub-family PAPILIONOIDEAE

Crotalaria assamica Benth.
Crotalaria incana L.
Crotalaria sericea Retz
Crotalaria usaramoensis Baker
Crotalaria anagyroides H.B. and K.
Desmodium uncinatum (Jacq.) DC.
Desmodium discolor Vogel
Desmodium barbatum (L.) Benth. and Oerst
Desmodium pabulare Hoehne
Desmodium cajanifolium DC.
Indigofera suffruticosa Mill.
Indigofera hirsuta L.
*_Erythrina indica_ Lam.
Erythrina poeppigiana Cook
Erythrina rubrinervia H.B. and K.
Erythrina arborea Small
Erythrina micropteryx Poepp.
Erythrina abyssinica Lam.
Erythrina cristagalli L.
Erythrina berteroana Urban
Erythrina velutina Willd.
Erythrina corallodendron L.
Erythrina monosperma Gaud.
Erythrina insignis Tod.
Erythrina fusca Lour.
Erythrina subumbrans (Hassk.) Merrill
Erythrina horrida Moc. and Sesse
Tephrosia candida DC.
Tephrosia purpurea (L.) Pers.
Tephrosia noctiflora Bojer
Andira inermis H.B. and K.
Piscidia erythrina L.
Stylosanthes guianensis Sw.
Vigna marina (Burm.) Merrill
Vigna owahuensis Vog.
Vigna sandwicensis A. Gray
Phaseolus lathyroides L.
Abrus precatorius L.
Alysicarpus vaginalis (L.) DC.
Alysicarpus longifolius W. and Arn.
Alysicarpus rugosus DC.
Canavalia campylocarpa Piper
Mucuna gigantea (Willd.) DC.
*_Pterocarpus indicus_ Willd.
Pterocarpus vidalianus Rolfe
Pterocarpus echinatus Pers.
Pterocarpus marsupium Roxb.
*_Lonchocarpus sericeus_ (Poir.) H.B. and K.
Lonchocarpus violaceous (Jacq.) H.B. and K.
Lonchocarpus domingensis (Pers.) DC.
Lonchocarpus latifolius (Willd.) H.B. and K.
Pongamia pinnata (L.) Merrill
Derris microphylla (Miq.) Val.
Clitoria ternatea L.

* Seeds of this species were used to test the nodule-forming abilities of the Rhizobia from other plants in this genus.

the tap root, whereas with the other species of trees nodule distribution tended to be more common on the lateral root mass.

With the exception of *Acacia baileyana* F. v. M. in the Mimosoideae and *Hymenaea courbaril* L. in the Caesalpinioideae, all of the plants listed in table 1 were moderately well nodulated. The species of Acacia, Inga, and Pterocarpus were the most heavily nodulated of the trees. Huge clusters of small and large nodules were generally distributed over the entire root systems. Leonard (8) has reported the rather general nodulation of Acacia species growing in greenhouse soil. Of 24 species (list not given) examined by him in 1924, only one, *Acacia baileyana* F. v. M., was found to lack nodules. Incidentally this species was, according to our observations, only sparingly inoculated. Several trees, each over 5 years old, were examined before nodules were found, and the positive cases noted showed only a few scattered, small nodules on very young tender roots. This was more or less unexpected, since species of Acacia are well distributed over the island, and, judged from the profuse nodulation on the other Acacia species, the root nodule organisms are equally so. On the other hand, nodulation on *Acacia decurrens* Willd. and *Acacia glaucescens* Willd. in the same general locality was exceptionally heavy.

Only one plant of *Andira inermis* H. B. & K. was available for examination; typical nodules, such as those to be noted in plate 1, figure 6, were abundant.

Nodulation of several species was of interest because of the nature and growth habits of the host plants. Nodules, averaging 10–20 mm. in length, were common on all the plants of *Piscidia erythrina* L. examined despite the fact that the root system of this species possesses Piscidin, an alkaloid ($C_{29}H_{24}O_8$) commonly used by natives in tropical areas to poison and stupefy fish. The roots were always accompanied by a most unpleasant and pungent odor. Likewise the root systems of various plants of *Derris microphylla* (Miq.) Val. were heavily nodulated. Various species of this genus are used for the extraction of rotenone.

Several variations in nodule types consistently appeared upon the root systems of species within a single genus. For instance, *Vigna marina* (Burm.) Merrill, a prostrate or climbing herbaceous annual vine common along the shore line, was always profusely nodulated despite the fact that the plants at high tide received heavy salt spray and (or) wetting by waves, and at low tide subsisted in dry sand. The nodules on this species were round, averaging 4–6 mm. in diameter, dull gray with smooth surfaces and with inky black centers. *Vigna owahuensis* Vog., a slender hispid indigenous vine common in the arid upland regions of the island, bore nodules practically identical with those commonly found on a well-inoculated clover root system. In like manner, the nodules on *Alysicarpus longifolia* W. and Arn. were tender and variously branched, similar to those on Crotalaria species, whereas those on *Alysicarpus vaginalis* (L.) DC. were round with rugged surfaces as is the type common on roots of *Vigna sinensis* L.

CROSS-INOCULATION STUDIES

Methods

The methods and techniques employed in the plant tests are given in the following detailed descriptions, since the infallibility of the results depends unquestionably upon the manner in which they were obtained.

Isolation of Rhizobia. At least five nodules were taken from the root systems of each species listed in table 1. Each nodule was washed under running tap water to remove the adhering soil, rinsed in three changes of sterile distilled water, and subjected to a violent agitation for 4–5 minutes in bichloride of mercury (1:1000). The nodule was then rinsed again in several changes of sterile distilled water, to remove the bichloride of mercury, and crushed in a tube containing 10 cc. of sterile water. Serial loop dilutions of this nodule suspension were then plated. In exceptional cases where the nodules were moderately large, a center portion of the nodule was removed for plating purposes with a sterilized needle.

Especial care was taken to avoid any probability of error due to mixed cultures or platings. Nodules of only one species were plated at one time, and, moreover, each nodule was considered a specific entity. Plates were labeled with the scientific name of the plant as well as with the index number of the species. This general procedure was followed throughout the series of isolations and pot experiments. Fresh mercuric chloride and rinse water in separate sterile dishes were used for each nodule, and, as a rule, all material pertaining to the previously plated nodule was removed from the laboratory table before the plating of another nodule was begun. In only a very few exceptional cases were nodules from more than two different species plated in one day.

Prior to Carroll's paper (3) Bacto yeast extract mannitol agar was used exclusively in the plating procedures. In the technique following this report asparagus extract mannitol agar was used in addition. On the whole this latter medium seemed preferable for many of the Rhizobia cultures, as the growth resulting from the nodule suspensions usually appeared in a shorter time. In such instances colonies suitable for isolation were usually apparent on the latter medium after 10 days' incubation at 25°C., whereas on the yeast extract mannitol agar plates suitable colonies were not apparent until 3–4 days later.

The incubation period of the cultures from the nodule suspensions varied considerably. The shortest time was required by the Rhizobia from the species of Erythrina. These colonies were usually discernible in 3–4 days and were large enough to warrant isolation in about 7 days; whereas the colonies from the nodules of the three species of Alysicarpus and from *Mucuna gigantea* (Willd.) DC. were not visible until about the fourteenth day and were not large enough to be isolated in less than 3 weeks.

At the time of isolation the colonies were examined with a Greenough-Leitz binocular microscope (6 X oculars), and only well-isolated colonies were picked

into yeast water mannitol broth. Growth as evidenced by turbidity was usually apparent after 7 days' incubation at 25°C. Replating and re-isolation of the cultures were repeated at least three times, and colonies were picked again in the manner described. The purity of the cultures was established by their reactions in litmus milk and by the characteristics of their growth on potato slants, on congo red and brom thymol blue yeast extract mannitol agars, and on asparagus extract mannitol agar. The cultural and physiological characteristics of these cultures will be reported at a later date.

Plant Tests. Verification of the isolated cultures as *bona fide* root nodule bacteria was achieved by rigidly controlled plant inoculation tests. Such tests were conducted in a special compartment of a greenhouse built in 1930. Only two sides of this compartment had outside exposure, although all sides had glass windows. The windows on the exposed side of the compartment were attached at the top by hinges and opened outward from the bottom on supporting rods. All air coming through these windows was filtered through a thin layer of cotton between two thickness of cheesecloth sewed to an inside window screen. The floor of this compartment was kept wet at all times, and the one door to this enclosure was closed except when in actual use as an entrance or exit.

The nodule-forming abilities of the newly isolated cultures were initially tested by inoculating either (*a*) seeds of their respective host plants or (*b*) seeds of a closely related species of the same genus. The latter method was used in many cases where it was not possible to obtain seeds from each of the respective species. Such examples have been designated by an asterisk in table 1. In several instances, such as with *Hymenaea courbaril* L. in the Caesalpinioideae and with *Andira inermis* H. B. & K. in the Papilionoideae, neither of the above procedures was possible, because viable seeds of these species could not be obtained locally or elsewhere. A slightly different obstacle was experienced with the seeds of the species in the genus Inga. Although a few seeds were obtained, all efforts to treat them satisfactorily were futile. In this genus the coats of the seeds resemble a soft wool-like mass from which the naked embryos escape and fall to the ground when the pods open. Various methods were employed to rid the embryos of the adhering microörganisms, but each resulted in killing the growing tips.

Seeds of the leguminous plants whose specificity of inoculation was to be tested were treated 3–4 minutes with 1:1000 solution of bichloride of mercury followed by 2–3 minutes of agitation in sterile distilled water heated to 60–64°C. They were then dried for several days and germinated in petri dishes on layers of sterile filter paper. Representative lots of the treated seeds were inoculated into nutrient broth and yeast water mannite broth to ascertain the efficiency of the treatment. Lack of microbial growth in the broths was taken to indicate an efficient treatment. In addition, seeds of *Samanea saman* (Jacq.) Merrill, *Albizzia lebbek* (L.) Benth., *Albizzia katangensis* Wildem., *Enterolobium cyclocarpum* Griseb., and *Mucuna gigantea* (Willd.) DC. were held in the open flame

after the mercuric chloride treatment until they were *slightly* charred. This method was resorted to with these seeds because natural germination in moist soil was found to require 6 months or more, and since efforts to scarify these hard, thick seed coats by ordinary means proved futile. Mortality as a result of the fire treatment was usually less than 10 per cent.

All plants were grown in sterile quartz sand contained in 1-gallon cans or in standard Mitscherlich pots. Prior to being filled with sand, the cans were painted with a non-toxic, rapid-drying, black paint to prevent rust. Several holes averaging about 4 mm. each in diameter were made in each can to afford drainage. The containers were filled with the dry quartz sand, and the tops were covered with thick wrapping paper and sterilized 8 hours on each of three successive days at 20 pounds pressure. Planting was made immediately after the third sterilization period.

The germinating seeds of the plants to be tested were placed on the moistened surface of the sand, and inoculation was made by adding several cubic centimeters of a 10-day-old broth culture of the organism in question. The seeds were then covered with sterile dry sand. The plants were watered as often as necessary with sterile distilled water and received sterile Crone's solution weekly.

Eight control pots were usually stationed at random on each table of 12 inoculated pots. Contamination was practically nil on the control series as observed by a close examination of the root systems of each plant. When the slightest contamination did occur, the data on the inoculated pots of the table or tables concerned were discarded.

Discussion and results of the plant tests

The classification of the tropical plant species into cross-inoculation groups followed two lines of approach. In the initial tests the newly isolated Rhizobia cultures from the tropical species were used as inocula for plants representative of the more common cross-inoculation groups, whereas in the later tests the reciprocal cross was attempted using as inocula Rhizobia cultures representative of the various plant groups upon seeds of the tropical species. The only exception to this two-fold procedure was the testing of the tropical seed against cultures of *Rhizobium japonicum* (Kirchner) Baldwin and Fred—an instance where lack of seed became an obstacle. However, in the counterpart of this particular series soybean and cowpea plants were grown in the same pots, both being inoculated simultaneously with the Rhizobia from the tropical species. In such experiments no occasion occurred wherein nodules were produced upon the soybean plants, whereas in every instance the root systems of the cowpea plants, intertwined with those of the soybean plants, were well nodulated.

Five stock laboratory strains of Rhizobia isolated from *Vigna sinensis* L. were used in the plant tests. Each of these strains is a beneficial inoculant, produces abundant nodules about the tap root systems of cowpea plants (*Vigna sinensis* L.), and has benefited the growth of cowpeas by the fixation of nitrogen

on repeated tests. These strains have also been used in cultures distributed for the inoculation of cowpea seed under field conditions.

Subsequent to establishing the nodule-forming abilities of the Rhizobia strains from the tropical leguminous species, five cultures were selected for use in the cross-inoculation tests, care being taken in each case to choose cultures representative of different nodules found on the plant species. A positive reciprocal cross-inoculation in the cowpea group occurred with each of the species listed in table 1, i.e., the strains of cowpea bacteria satisfactorily inoculated and enhanced the growth of the tropical plant species, and in turn the Rhizobia isolated from the nodules of the tropical leguminous species were beneficial to cowpea plants. The word "positive" may be taken to indicate a minimum of 15 nodules per plant, which was in every case accompanied by an appreciable fixation of nitrogen in comparison with the uninoculated plants.[3] Therefore, in the opinion of the authors the results obtained warrant the addition of 87 plant species to the cowpea cross-inoculation group, provided *Vigna sinensis* L. is accepted as an indicator test plant for that group. Four of the species in table 1; namely, *Crotalaria incana* L., *C. anagyroides* H. B. and K., *C. usaramoensis* Baker, and *Clitoria ternatea* L. were included in the Carroll list (3). Of the new additions reported in this paper, 72 species are trees and shrubs, and the remaining 15 species are herbs and vines.

The conclusions from these plant tests have been based upon three replicative greenhouse experiments in which each culture was run in triplicate.

Tropical species versus inoculation. All of the tropical plant species tested were inoculated by their own Rhizobia strains respectively, as well as by the cultures of cowpea bacteria. In a few exceptional cases, such as with *Samanea saman* (Jacq.) Merrill and the species of Erythrina, the host strains seemed to be more beneficial on their respective host plants as evidenced by the abundance and type of nodules produced and by the growth and color of the plants.

Characteristic differences in color of the plants and in luxuriance of growth occurred in all cases between the inoculated and the uninoculated tropical species. Such differences were more pronounced with the plants in the subfamily Papilionoideae. With the exceptions of *Mucuna gigantea* (Willd.) DC. and *Pongamia pinnata* (L.) Merrill, the seeds of the plants in this sub-family were relatively small, and therefore a stunted growth occurred as a result of nitrogen hunger in the uninoculated plants. The response to inoculation by members of this sub-family was very satisfactory. On the other hand, the seeds of the species in the Mimosoideae and Caesalpinioideae were considerably larger, and much growth occurred before nitrogen hunger appeared (in the control series). With several of the species in these sub-families, such as *Enterolobium cyclocarpum* Griseb. and *Parkia javanica* (Lam.) Merrill, it was believed that pot-binding inhibited the maximum differences in growth that would have resulted. It appeared that some of the Albizzia species were not

[3] For the sake of brevity, nitrogen analyses of the plants by the Gunning-Kjeldahl method have been omitted in this report.

suited to sand culture, since they grew very slowly and attained a height of only 8–10 inches in a 3-month period. Marked distinctions in color, however, were evident, the controls being very yellow, losing leaflets, and becoming bare, whereas the inoculated plants maintained a dark green appearance yet showed a stubby growth.

Plate 1, figure 1 shows an example of the results obtained by the inoculation of seeds of *Enterolobium cyclocarpum* Griseb. with the host strain 34-7 and with cowpea 10. The control plants were very yellow, and the inoculated plants a dark green. The finely pinnate leaf system of the species accounts for the droopy appearance of the plants. Figures 2 and 3 of plate 1 show the root system of one plant from each of the inoculated pots. Each plant of *Enterolobium cyclocarpum* Griseb. inoculated with strain 34-7 averaged 76 nodules per plant and 4.0 gm. dry weight, whereas each of the plants inoculated with cowpea strain 10 approximated 100 nodules but only 3.1 gm. dry weight. The control plants were without nodules, showed evidences of nitrogen hunger, and averaged only 1.8 gm. dry weight per plant. Figures 1, 2, and 3 of plate 2 show somewhat similar results obtained when seeds of *Erythrina indica* Lam. were inoculated, although the Erythrina cultures, as evidenced by numbers and types of nodules and by color and dry weights of the plants, were more effective on the plants of *Erythrina indica* than were cowpea cultures.

It is not within the scope of this report to deal with the various examples of host specificity and strain variation observed, since the primary purpose of this study was merely to ascertain the status of cross-inoculation. Mention should be made, however, that the cultures isolated from *Samanea saman* (Jacq.) Merrill showed an exceptional example of host specificity. All of the Samanea cultures, as well as the cowpea strains, beneficially inoculated plants of *Samanea saman* (Jacq.) Merrill as evidenced by the color and growth and by the type of nodules on the Samanea roots, whereas, the cowpea plants when inoculated with the Samanea cultures produced only small well-scattered nodules, and only slight differences in growth occurred between the control and treated plants. By analysis the cowpea plants inoculated with the *Samanea saman* cultures averaged 2.9 per cent nitrogen in comparison with an average of only 1.21 per cent nitrogen in the control cowpea series. Additional tests showed the same general type of results when the Samanea cultures were used to inoculate seeds of *Acacia koa* A. Gray, *Cajanus cajen* (L.) Millsp., and *Crotalaria juncea* L. Figure 4, plate 2, shows the type of nodule produced on pigeon pea plants [*Cajanus cajan* (L.) Millsp.] by cowpea strain 10, and figure 5, plate 2, illustrates the type of nodule production by the strains of *Samanea saman* (Jacq.) Merrill.

Although Rhizobia were not isolated from nodules of the following species, it seems quite logical to assume that these species belong to the cowpea group, since plants of each were inoculated with cowpea nodule-forming bacteria. It might be fitting, however, that definite inclusions of these species within the cowpea group await data pertaining to the inoculation of cowpea plants with

these specific Rhizobia strains. In this regard, Rhizobia cultures of *Acacia koa* A. Gray, strains 15-3 and 15-4, and cowpea strains 10 and 12 satisfactorily inoculated seeds of *Acacia koa* var. *lanaiensis* Rock, *Acacia koa* var. *hawaiiensis* Rock, and *Acacia kauaiensis* Hillbr. Likewise cowpea cultures 10 and 12 and strains 9-2 and 9-4 of *Crotalaria sericea* Retz satisfactorily inoculated seeds of the following species of Crotalaria obtained from various experiment stations:

C. cunninghamii R. Brown
C. sphaerocarpa Perrott
C. natalitia Meisn.
C. astragalina Hochst.
C. fulva Roxb.
C. longirostrata Hook. and Arn.
C. tetragona Roxb.
C. grantiana Harvey

Present status of the cowpea group. At the present time two detailed summarizations of the cowpea group appear in the literature, one by Walker (19) in 1928, the other by Fred, Baldwin, and McCoy (4) in 1932. It becomes apparent from a comparison of the two summarizations that the latter compilation is the more accurate; thus a total of 21 genera and 41 species constituted the nucleus to which Carroll in 1934 (3) added 30 species comprising 8 genera. Five of the latter genera, Apios, Aeschynomene, Erythrina, Mimosa, and Clitoria were new to cross-inoculation grouping.

The data presented in this report warrant, in the opinion of the authors, the addition of 30 species of 16 new genera and 57 species of 10 previously placed genera to the cowpea group. These results were obtained by the inoculation of seeds of the tropical species with cowpea nodule bacteria and the reciprocal inoculation of cowpea seed by the newly isolated Rhizobia from these tropical species. Three exceptions to this procedure have been noted in the preceding discussion, i.e., species of Inga, Hymenaea, and Andira.

Since the establishment of the cowpea group, additional experimental evidence has shown it to consist of a versatile and cosmopolitan collection of leguminous plants. Burrill and Hansen (2) in 1917 were probably the first to note this dissimilarity to the other cross-inoculation groups. In the past 18 years the addition of various herbs, vines, shrubs, and trees have served to make the versatility of the group more obvious.

It is generally accepted that a cross-inoculation group implies a group of plants in which the nodule-forming bacteria are mutually interchangeable. Whether the now so-called "cowpea group" complies with this definition might well be worthy of experimental consideration. In practically all of the evidence heretofore offered, only *Vigna sinensis* L. and the nodule bacteria therefrom have been used as indexes for the addition of species into the group, whereas the smaller number of species in the other groups have permitted a greater range of host tests.

Recently Carroll (3) has shown some interesting data concerning irregularities in nodule formation by cowpea bacteria on supposedly interchangeable host plants within the cowpea group. He reported several cases wherein host plants accepted as *bona fide* members of the cowpea group failed to produce nodules when inoculated with Rhizobia from other plants in the same group. In practically all instances a greater number of nodules occurred on the roots of *Vigna sinensis* L. than on the other host plants regardless of the source of inoculum. Such irregularities are so far without explanation.

Various investigators have emphasized the close relationship existing between the root nodule bacteria of the cowpea and soybean groups. As early as 1917 Burrill and Hansen noted the similarity in growth characteristics of the bacteria from the two groups. Others have confirmed these similarities. In 1921 Löhnis and Hansen (10) and Shunk (17) showed that the cowpea and soybean bacteria were similar in that they possessed monotrichous flagella, whereas the bacteria from the other groups were peritrichously flagellated. Similarity in reactions has also been noted in litmus milk (10).

According to Sears and Carroll (16), C. D. Jones was the first to show a kinship in nodule-forming ability between the two groups: his results showed that a culture of soybean nodule bacteria produced nodules upon cowpea as well as upon soybean roots. Since then Leonard (7), Sears and Carroll (16), Hansen and Tanner (6), Walker and Brown (20), and Carroll (3) have cited additional evidence showing that such interchangeability in nodule-forming function does occur. This interchangeability, however, has not been perfect in all cases. In the majority of instances soybean cultures have possessed the power to induce nodules on the roots of cowpea, whereas only a few of the cowpea cultures tested have infected the soybean plant. Recently Walker and Brown (20) have proposed that the root nodule bacteria of the cowpea and soybean groups be considered as the one species now generally recognized as *Rhizobium japonicum* (Kirchner) Baldwin and Fred.

Serological tests have given enlightening evidence on the cross-inoculation status of various plants but have not been free of incongruous results. The recent contribution of Hansen and Tanner (6) has shown that the correlation between cross-inoculation and cross-agglutination is not so direct as might be inferred from the work of Simon (18). Yet a relation has been shown to exist between serological behavior and the cross-inoculation phenomenon, despite the fact that it is not a reciprocal one. Whether serological methods may become basic in cross-inoculation controversies seems questionable. Recently results have shown that all strains from a single cross-inoculation group did not cross-agglutinate. Hansen and Tanner (6) have recently shown six cultures from the cowpea group to fall into three agglutination groups, five cultures of which were isolated from cowpea plants. Seven soybean cultures were divided into two agglutination groups. In addition two strains of cowpea bacteria cross-agglutinated with two strains of the soybean organism. Thus, more than

one serological strain has been isolated from the same host, yet no two serologically similar nodule-forming bacteria from different host plants have been found which failed to cross-inoculate.

Inasmuch as the criterion for species differentiation within the genus Rhizobium has been the ability to inoculate plants, the question of host affinities becomes of greater significance. It seems questionable that the evidence to date warrants the consolidation of the soybean and cowpea groups under one species of Rhizobium. In some cases the numbers of nodules produced on plants in the cross-inoculation tests have been too few to warrant the importance attributed to them. In other cases plus signs have been used to indicate the extent of nodulation, but explanatory remarks have not elucidated whether the plus signs signified one nodule or two dozen nodules. Although it is admitted that too much significance cannot be placed upon the actual numbers of nodules produced on a plant by a given culture, yet, in instances of this nature, numbers are of more value than symbols. Whether the soybean bacteria benefit cowpea plants and *vice versa* may be a technicality aside from the main issue of the ability of the bacteria to cross-inoculate, but it is decidedly pertinent from an agricultural viewpoint. Data of this nature have not been presented. Moreover, the small number of soybean and cowpea cultures and plants tested to date by the various authors should not be taken to represent the far greater number of plants involved. A greater variety and number of host plants and Rhizobia, along the line previously presented by Carroll (3), need to be tested. Before the consolidation of the two groups becomes generally recognized, interchangeability of cowpea and soybean bacteria on the host plants of the two groups should affect a normal occurrence and not exceptions to the general rule.

Whether the consolidation of the soybean and cowpea groups is a logical move at this time is doubtful. It is very likely that the cowpea group, now so very large and unwieldy as a unit in the sense of the present definition of a cross-inoculation group, could better be subdivided into smaller or sub-groups based on susceptibility to certain strains of Rhizobia from plants more or less closely related, and yet maintain some degree of unity. It is very probable that the soybean plant would bear a close relationship to certain ones of these groups.

SUMMARY

The data in this report have dealt with observations on nodule formation of various tropical leguminous plants and cross-inoculation tests with these species.

The versatility of the now so-called "cowpea group" has been further emphasized by the addition of various distantly related leguminous plants as a result of rigidly controlled plant inoculation tests.

The present status of the cowpea group is briefly discussed.

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PLATE 1

FIG. 1. Response of *Enterolobium cyclocarpum* to inoculation.

Left, control, no inoculation. Center, inoculated with strain 34-7, isolated from a nodule of *Enterolobium cyclocarpum*. Right, inoculated with strain 10, isolated from *Vigna sinensis*. Age of plants, 3½ months.

FIG. 2. Nodule formation on *Enterolobium cyclocarpum* inoculated with strain 34-7.

FIG. 3. Nodule formation on *Enterolobium cyclocarpum* inoculated with cowpea strain 10.

FIG. 4. Nodules from *Acacia horrida* growing in forest soil. One-half natural size.

FIG. 5. Nodules from *Inga edulis* growing in field soil. One-half natural size.

FIG. 6. Nodules from *Andira inermis* growing in forest soil. One-half natural size.

FIG. 1

FIG. 2

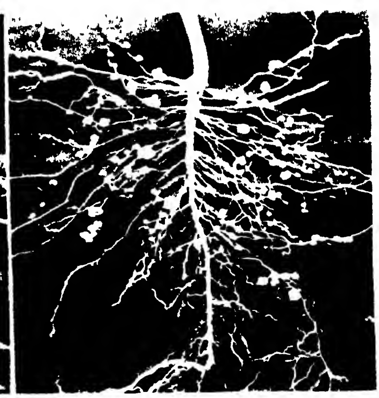

FIG. 3

FIG. 4

FIG. 5

FIG. 6

PLATE 2

FIG. 1. Response of *Erythrina indica* to inoculation.

Left, control, no inoculation. Center, inoculated with strain 36-4 isolated from a nodule of *Erythrina indica*. Right, inoculated with strain 10 isolated from *Vigna sinensis*. Age of plants, 3 months.

FIG. 2. Nodule formation on *Erythrina indica* inoculated with strain 36-4.

FIG. 3. Nodule formation on *Erythrina indica* inoculated with cowpea strain 10.

FIG. 4. Nodule formation on the roots of *Cajanus cajan* inoculated with cowpea strain 10.

FIG. 5. Nodule formation on the roots of *Cajanus cajan* inoculated with strain 13-5 isolated from a nodule on *Samanea saman*.

FIG. 1

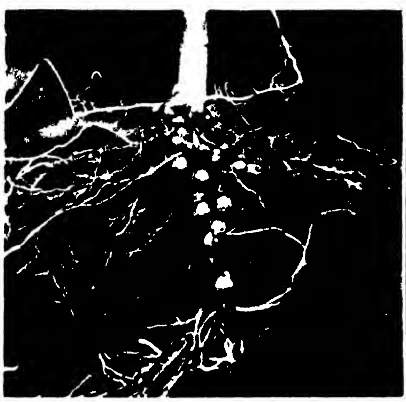

FIG. 2

FIG. 3

FIG. 4

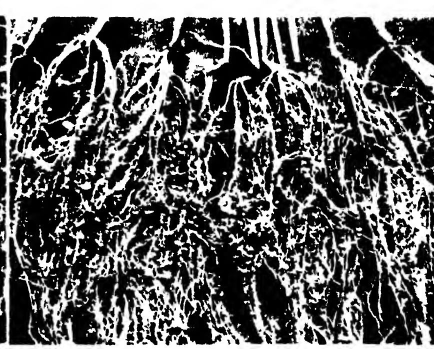

FIG. 5

GEORG WIEGNER

Georg Wiegner
1883-1936

"Young friend," Prof. Georg Wiegner once said to one of his assistants, "when I am dead you will have to write my obituary, and remember, my creed is that of duty, as taught by Kant." With a joke, the young assistant changed the subject but promised to write a paper on "Professor Wiegner" with the reservation that he could write as he pleased. This incident was promptly forgotten until ten years later, when, on April 14, 1936, a cable, "Professor Wiegner died this morning," brutally reminded the present writer of his promise.

CURRICULUM VITAE

Georg Wiegner was born in Leipzig, Germany, on April 20, 1883, the third of twelve children. His youth was clouded with hardship and poverty. He told me once that because he could not afford to buy a Latin dictionary he had to copy one by candle light. With the aid of far-seeing teachers and friends he was permitted to attend the Realgymnasium, which he finished as Primus Omnium in 1902. In the same year he enrolled in the University of Leipzig and graduated in chemistry in 1906. His thesis on "Metastable conditions between solid and gaseous substances" already revealed the keen thinker and successful investigator. From October 1906 to October 1907 he served in the army and then accepted a position as assistant chemist at the Agricultural Institute of the University of Göttingen. His experience at this famous center of learning proved to be a turning point in his life because here he was drawn to the great Zsigmondy, co-inventor of the ultramicroscope, who "captured" Wiegner for colloid chemistry. Soon Wiegner handled the ultramicroscope like no one else. In 1911 he became Privatdozent for Agricultural Chemistry at Göttingen, and in 1913 he married Katharina Claus. In the same year he was offered a professorship in agricultural chemistry at the Federal Institute of Technology in Zürich, Switzerland, which, fortunately for the Swiss, he accepted. Here began his ascending path, which in the course of 23 years led him to international fame. The World War temporarily interrupted Wiegner's climb. He was wounded before the English lines, but in 1916 returned to his friends in Zürich. He remained faithful to his chosen country and declined attractive calls to the Universities of Bonn-Poppelsdorf, Hohenheim, and Munich (as successor to Ramann).

Wiegner became an honorary member of many learned societies. On account of family traits Wiegner never expected to live long. With this in view he carefully trained his body with exercise and sports and appeared a picture of health. Suddenly on April 2 of this year a serious stomach opera-

tion became necessary, which, quite unexpectedly, he did not survive. He was 53 years old. He had no children.

THE LAST UNIVERSAL AGRICULTURAL CHEMIST

Liebig applied chemistry to agriculture, and for him the soil, the plant, and the animal formed a closed and inseparable cycle. Today agricultural chemistry is becoming so highly differentiated into soil phases, plant chemistry, animal nutrition, dairy chemistry, fertilizer production, etc., that it becomes increasingly impossible for a human being to master them all. Wiegner's genius appeared just at the right moment to accomplish this feature for the last time in the history of science. The extent of his vast field of learning is evident from the courses he regularly taught: quantitative agricultural chemistry; soil science; fertilizers, their production and use; animal nutrition, introductory and advanced; chemistry of milk and milk products; chemistry of feeding stuffs; agricultural technology; colloid chemistry. He lectured on soil science every semester, either for agricultural students or for foresters. On certain days he lectured five hours. Every Monday afternoon he took his class on a field trip in coöperation with the Crops Department.

The surprising thing is that Wiegner not only delivered his lectures in admirable form, but that he was an international authority in every field he taught. Soil science, nutrition, dairy science, and colloid chemistry all claim him as a leader in their respective disciplines. He was made an honorary member of the International Society of Soil Science (1935); he was given an honorary degree in veterinary science for his work in nutrition (1933); and the International Colloid Society awarded him the Laura Leonard medal for outstanding research in theoretical and applied colloid chemistry (1934).

His interest oscillated somewhat irregularly among the various branches of agricultural chemistry, as revealed by his publications. He wrote about a dozen papers on pure physical and colloid chemistry, ten papers on colloid chemistry of milk, nearly forty papers on soils, and over thirty papers on animal nutrition. He published well over a hundred articles, not including the dissertations of his students and the papers of his coworkers.

Wiegner started out in physical chemistry. At Göttingen his chief, Professor Fleischmann, aroused his interest in milk chemistry, and Wiegner published a series of papers on milk sugar, milk serum, specific gravity of milk, and degree of dispersion of milk constituents. Professor Baragiola once remarked that with Wiegner a new era had begun in dairy chemistry.

During his assistantship Wiegner took agricultural courses and became fascinated with chemical problems in soils, particularly with base exchange. His splendid contributions in the domain of soils will be dealt with in a separate chapter.

Professor Lehmann introduced Wiegner into the science of nutrition, but the young Privatdozent postponed special investigations until his professorship at Zürich. As a consequence of food scarcity in Switzerland during the War,

Wiegner attacked the problems of digestability of war bread and chemically treated foodstuffs. His equipment was most primitive. He had a few rabbits and one ram. His mode of approach and the results obtained, however, were so impressive that the Swiss farmers gained confidence in Wiegner and in 1925 provided him with a well-equipped institute of animal nutrition. He trained an excellent staff of assistants and studied both practical and theoretical phases of animal nutrition. His contributions to the theory of nutrition, the mathematical formulation of feeding effects, and the energy evaluation of foods on the basis of meat and fat production have attracted world-wide attention. Much emphasis was placed on methods of conservation of silage and on the merits of various forms of hay making. In his pasture studies Wiegner had a fine opportunity to combine his knowledge of soils, of grass quality, and of animal nutrition.

In 1926 Wiegner published a laboratory manual on quantitative agricultural chemistry, dealing with analyses of soils, fertilizers, feeds, milk, and wine (Borntraeger, Berlin, 1926, 348 pages).

If Liebig was the father of agricultural chemistry, Wiegner was the last of the generation as far as supreme command of the entire field is concerned. In looking back upon Wiegner's life and impressive production, many points of contact between Liebig and his admirer, Wiegner, appear. There is great similarity not only in the types of problems studied but also in their viewpoints on the value of science in agriculture. Like Liebig, Wiegner disliked the utilitarian intruders in agricultural science who worshipped the god of production. "Judging a piece of research merely by its immediate practical value may be excused for an experiment station," Wiegner once said, "but at the University the agricultural chemist should assume a broader outlook and strive for fundamental truth."

WIEGNER AS A SOIL SCIENTIST

Wiegner was not a "soils man" as we understand the term in this country. He had no agricultural background and had never plowed a field. His contact with the soil was indirect. He was a chemist, and the soil was an object through which colloid chemistry might be studied and applied. He was not interested in the soil as such. In this respect he was a counterpole to Marbut.

In spite of his handicap—or perhaps because of it—Wiegner has influenced the development of modern soil chemistry more than any other contemporary soil scientist, Gedroiz perhaps excepted. If soil science is to come of age Wiegner will have played no small part in it. For a century soil scientists have unscrupulously borrowed from the pure sciences, as a matter of course, without thinking of repaying anything. Wiegner started to pay back. His sedimentation apparatus was welcomed in many scientific and industrial laboratories. He showed that base exchange is not a mere soils problem; rather it is a general principle in nature. Biochemists and chemists in general, have slowly begun to realize the truth of Wiegner's contention. Wiegner's principles of coagula-

tion, their theory and application, are a permanent ornament to soil science. If we want to have a soil science with emphasis on *science* we must do more than help the farmer: we must contribute to the general store of knowledge, discover laws, invent methods, and produce theories which also fertilize other sciences. Wiegner has blazed the trail in this direction. So has Marbut. Both thought highly of each other.

Wiegner's first contribution to soil science has become a classic. It was published in the *Journal für Landwirtschaft*, volume 60, 1912, under the title "On base exchange in soil." Wiegner gave credit to the work of Thomas Way and showed that Liebig had obscured the issue. According to Wiegner base exchange is a chemical reaction as far as equivalency is concerned, but otherwise has all the characteristics of an adsorption reaction. It follows Freundlich's isotherm.

During the War, in the trenches, Wiegner conceived his well-known sedimentation apparatus. For decades soil scientists had been content with the isolation of certain soil fractions, such as sand, silt, and clay, but Wiegner, as a colloid chemist, wanted to know the entire distribution curve. Like the Sven Oden balance, the Wiegner tube gives a continuous distribution diagram and for certain purposes is superior to other methods. The apparatus has been modified by several investigators.

In 1916 Wiegner gave a lecture on soils before the Society of Natural Sciences in Zürich, as a result of which he published a little book entitled "Soil and soil formation from a colloid chemical standpoint" (Steinkopf, Dresden, sixth unrevised edition, 1931). This brief but excellent exposition at once made Wiegner popular and internationally known. Here was something new. The common idea of the soil's containing mysterious colloids was changed to the concept of the soil as a disperse system. Clay formation was explained as a mutual flocculation of negative, colloidal silicic acid and positive, colloidal aluminum and iron hydroxides.

From 1922 to 1927 Wiegner was particularly active in soil research. In a paper "Dispersity and base exchange" (Zsigmondy volume of the *Kolloid Zeitschrift*, 1925) he laid the cornerstone of modern research on soil colloids and on certain phases of pure colloid chemistry as well. In the introduction he wrote:

> In my opinion base exchange is a problem which embraces more than the special field of soil science. It is a part of general dispersoid chemistry. Base exchange is of fundamental importance not only for the cation content of clay, permutite, and soil—in which soil scientists are particularly interested—but is also determinant for the qualitative and quantitative properties such as stability, swelling, etc., and for the variations of properties (peptization and flocculation) of most colloidal systems (colloidal sulfur, AgCl, As_2S_3 dye stuffs, proteins, etc.).

In the same paper he introduced into soil chemistry the concept of hydration of ions in order to explain the lyotropic series. Subsequent detailed experiments on base exchange with permutite demonstrated the fruitfulness of this

approach. Coagulation studies, viscosity measurements, heat of wetting, and and swelling data, all proved the significance of the nature of the adsorbed ion.

In connection with the sedimentation apparatus Wiegner started to re-investigate the mechanism of coagulation. He distinguished between orthokinetic and perikinetic flocculation and, with the aid of the mathematical physicist H. Müller, put the Smoluchowski equations on a broader foundation. Smoluchowski's formulas apply only to monodisperse systems (e.g., colloidal gold, or Sven Oden sulfur), whereas the new equations comprise polydisperse systems such as soil.

About 1930 Wiegner published the suspension effect which was to change radically our notions of soil acidity and alkalinity. He showed that the adsorbed H ions affect the electrode, an observation previously made and explained by Bradfield. Wiegner extended the same concept to adsorbed OH ions. An excellent summary of his viewpoints has been published in English in the *Journal of the Society of Chemical Industry*, volume 50, pp. 55–62, 65–71, 103–112, 1931, under the titles "Coagulation" and "Some physico-chemical properties of clays."

Wiegner took an active though indirect part in the soil survey of Switzerland and directed extensive studies on the destruction of concrete pipes in soils.

Professor Wiegner once remarked that in science every man eventually returns to his first love. He entered soil chemistry by the way of base exchange and left it by the same avenue. His last publication was on "Ionic exchange and structure," which appeared in the *Transactions of the Third International Congress of Soil Science*, volume III, p. 5–28, 1936. A comparison between Wiegner's first and last papers affords a good illustration of the impressive development of a research problem in the hands of a great sculptor in science.

WHAT MADE WIEGNER A GREAT SCIENTIST?

Three important features have contributed to Wiegner's success: he possessed a wealth of original ideas; he was thoroughly trained and was *gründlich* in his work; he knew how to present his results.

The first attribute cannot be acquired. Wiegner was given a full share of it. He had new ideas about everything. No matter what was discussed, be it science, philosophy, politics, America, dictators, or what not, he surprised his listeners with new angles, original viewpoints, and unsuspected cause and effect relationships. He was particularly prolific in the production of scientific ideas and he saw problems everywhere. At one time he had nearly twenty associates and kept every one of them at a high pitch with daily discussions and stimulating interpretations. He was a born general in the field of research.

Wiegner went through the old German educational mill—hard work in school and more work at home; memorizing and analyzing. It cleared the head and gave a solid foundation. Wiegner left the Realgymnasium as the highest ranking student and he forgot nothing of what he had learned. As the "Old Professor," he still beat everyone in multiplication and division (no machines

needed); complicated mathematical formulas seemed ever ready up his sleeve. He remembered the classics better than did his students who came directly from the school bench. At the university he had grand teachers, the greatest in German science, Nobel prize winners left and right. He saw into their workshops and learned his lesson. "Good training in the past is not sufficient," Wiegner asserted, and he kept up with modern developments. At the height of his fame, he attended Debye's courses on electrolytes and took courses in advanced mathematics. He used to say that a chemist has to relearn chemistry about every ten years. He did it—at night. Like Willstätter, he could have said: "For thirty years I have heard the midnight bells at my desk."

The Germans are thorough and systematic, and Wiegner was a typical example. He checked and rechecked his measurements, he calculated backwards and forwards. I remember the time when I collaborated with him in preparing a textbook on agricultural chemistry. We wished to discuss pH measurements. What is the proper soil-water ratio? He made me go through all the German, French, English, Dutch, and Scandinavian literature and tabulate what others suggested. Then I had to test the formulas in the laboratory. Naturally, it took many days to write a page, but then the material stood the test.

Wiegner was an artist also. True, he cared little for music, painting, or sculpture, yet he was a born poet and at times liked to compose. He could not help but reveal this trait in his scientific papers. He never published a mere progress report, a little contribution, or a note to the editor. He presented a finished whole, a dramatic account of the problem, thesis and antithesis, attractive descriptions. His scientific papers read like novels and his publications always made a hit. No one will forget his speeches. When Wiegner rose, the atmosphere became electrified, people listened most intently, and a roaring applause followed his finale. On such occasions we assistants were particularly proud of our master and admired him accordingly.

Wiegner has trained a formidable array of research workers. Not counting the pure colloid chemists and nutrition men, such soil scientists have been associated with Wiegner, for longer or shorter periods, Albareda, R. V. Allison, Barnette, Beutelsbacher, Blom, Bradfield, Cernescu, Gallay, Georgescu, Gessner, Jenny, Kawashima, Marshall, A. Meyer, K. W. Müller, Oedelien, Pallmann, Renold, E. W. Russell, Salminen, Scherf, Steward, Souviron, Szigeti, Tendeloo, Tokuoka, Tuorila, Weisz. The list is by no means complete.

WIEGNER AS A TEACHER AND FRIEND

Certain people are of the opinion that a good research man necessarily is a poor teacher, or, *vice versa*, that a good teacher makes a poor scientist. Anyone acquainted with Wiegner will gladly testify to the contrary. Wiegner was a great scientist as well as a gifted teacher. He never had a course in education, yet his lectures were a thrill to his students. They did not miss a single lecture, and this is the crucial test in an educational system in which attendance is not enforced.

Whoever entered Wiegner's laboratory as a co-worker found the door open to his private life. He gave himself unmasked and freely expressed his opinions about the outer world. By this he gained the confidence of his associates, and we told him our personal troubles and asked for advice, which he freely gave. Looking back to the twenties, I am now ashamed to recall how much of his precious time we consumed with our own petty affairs, even going so far as to read to him passages from Schopenhauer, who at that time had our attention. Wiegner did not stop us or complain; in fact, he lived with us because he had remained young. Like all great men and really busy people he seemed to have time for everything and everybody.

Wiegner's body is gone. His discoveries will become commonplace, his theories revised, and his methods changed beyond recognition; but his spirit is preserved in the hearts of his students and will be passed on from generation to generation.

HANS JENNY.

PLANTS IN THE SUB-FAMILY CAESALPINIOIDEAE OBSERVED TO BE LACKING NODULES[1]

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The symbiotic relationship between leguminous plants and the root nodule bacteria is commonly recognized by the formation of nodules on the root systems. The majority of plants attributed this characteristic to date are members of the sub-family Papilionoideae, although nodule formation is not uncommon in the Mimosoideae. Many exceptions are known to occur in the Caesalpinioideae.

Cassia chaemacrista L., added to the cowpea group by Burrill and Hansen (7) in 1917, is the only species of the sub-family Caesalpinioideae contained in the cross-inoculation groups compiled by Walker (23) and by Fred, Baldwin, and McCoy (11). Recently additional species, *Cassia mimosoides* L., *Cassia pilifera* Vog., and *Hymenaea courbaril* L., have been added to the same group (2). Rhizobia cultures isolated from nodules of each of these species inoculated cowpea plants. It was possible, however, to make the reciprocal cross with only two of the species, *Cassia mimosoides* L. and *Cassia pilifera* Vog., with cowpea bacteria, since seeds of *Hymenaea courbaril* L. could not be obtained.

Various observations on the lack of nodule formation on species of the Caesalpinioideae have been reported. A list of such species appears in table 1.

During the past 5 years a rather extensive survey of nodule formation has been conducted on tropical leguminous plants growing in the Territory of Hawaii. Special attention was directed to the species of the sub-family Caesalpinioideae in an attempt to understand better the extent of nodulation. Table 2 contains the data obtained from the survey.

All of the plants examined were growing under natural conditions in forest or field soil. Seeds of practically all of the species were imported sometime in the distant past from other tropical areas. The plants have become well established, many now existing as large trees. Examination consisted of two procedures: (*a*) the removal of the soil from most of the root mass and (*b*), in many instances, the planting of seeds of the species in several lots of island soil known to contain Rhizobia representative of the cross-inoculation groups. The latter is true with all species listed in table 2 of which more than four plants were examined. It is questionable that data obtained from such a

[1] Published with the approval of the director as Technical Paper No. 91 of the Experiment Station of the Pineapple Producers' Cooperative Association, University of Hawaii.

TABLE 1

Members of the sub-family Caesalpinioideae reported to be lacking nodule formation

SCIENTIFIC NAME (COMMON NAME)	AUTHORITY
Cercis canadensis L. (Redbud or Judas tree)	Lachmann (16), 1858; Buckout (5), 1889; Harrison and Barlow (13), 1906; Leonard (17), 1925
Cercis siliquastrum L.	Clos (9), 1893
Gymnocladus dioica (L.) Koch (Kentucky coffee tree)	Buckout (6), 1890; Leonard (17), 1925; Harrison and Barlow (13), 1906
Cassia barclayana Sw.	Clos (9), 1893
Cassia marylandica L. (Wild or American senna)	Leonard (17), 1925
Cassia medsgeri Shafer (Medsger's wild senna)	Leonard (17), 1925
Cassia tora L. (Low senna, coffee-weed)	Leonard (17), 1925
Cassia occidentalis L. (Coffee senna, styptic-weed)	Leonard (17), 1925
Cassia corymbosa Lam.	Leonard (17), 1925
Cassia bicapsularis L. (Stiber-bush)	Leonard (17), 1925
Cassia emarginata L.	Leonard (17), 1925
Cassia laevigata Willd.	Leonard (17), 1925
Cassia tomentosa Wall.	Leonard (17), 1925
Cassia artemisoides Gaudlich	Leonard (17), 1925
Gleditsia triacanthos L. (Honey locust)	Nobbe, Schmid, Hiltner and Hotter (21), 1891; Harrison and Barlow (13), 1906; Leonard (17), 1925
Caesalpinia brevifolia Baill.	Naudin (20), 1894
Caesalpinia exostemma Moss. & Sesse	Leonard (17), 1925
Caesalpinia melanocarpa Griseb.	Leonard (17), 1925
Caesalpinia pulcherrima (L.) Sw. (Barbados pride, dwarf poinciana)	Leonard (17), 1925
Ceratonia siliqua L.	Leonard (17), 1925

TABLE 2

*Tropical species of the sub-family Caesalpinioideae observed to be lacking nodules**

SCIENTIFIC NAME (COMMON NAME)	HABITAT	KIND OF PLANT	NUMBER EXAMINED	AGE OF PLANTS EXAMINED
Saraca declinata Miq.	Sumatra	Tree	3	About 3 years
Saraca thaipingensis Cantley	China and Malay	Tree	3	About 3 years
Saraca indica L. (Asoka, sorrowless tree)	East Indies and Malay	Tree	3	About 3 years
Schotia brachypetala Sond.	South Africa	Shrub	15	1–5 months
Schotia latifolia Jacq. (Elephant hedge or bean tree)	South Africa	Tree	3	About 3 years

* The authors are deeply indebted to Mr. E. L. Caum, assistant botanist of the Experiment Station of the Hawaiian Sugar Planters' Association, for his making available the majority of the species listed in this table and for his generous aid in their classification.

TABLE 2—*Concluded*

SCIENTIFIC NAME (COMMON NAME)	HABITAT	KIND OF PLANT	NUMBER EXAMINED	AGE OF PLANTS EXAMINED
Tamarindus indica L. (Tamarind)	Tropical Africa	Tree	16	12 seedlings 1–4 months, 4 trees about 7 years
Bauhinia binata Blanco	India	Small tree	20	3 months to 1 year
Bauhinia monandra Kurz.	Burma	Small tree	15	3 months to 1 year
Bauhinia variegata Linn (Napoleon's hat, mountain ebony)	East Indies and Burma	Tree	38	3 to 5 months
Cassia fistula Linn (Indian laburnam, golden shower)	Tropical Asia	Tree	12	2 months to 2 years
Cassia grandis L f (Horse cassia, liquorice tree, pink shower)	South America	Tree	22	1 month to 2 years
Cassia laevigata Willd. (Smooth senna)	Tropical America	Shrub	40	1 month to 2 years
Cassia glauca Lam	India	Small tree	50	1 month to 5 years
Cassia nodosa Burch.-Ham. (Pink and white shower)	Tropical Asia	Tree	18	2 months to 2 years
Cassia occidentalis Linn (Negro coffee, stinking weed, common cassia)	Tropical America	Shrub or herb	Several hundred	1 month to 2 years
Cassia moschata H B & K	South America	Tree	4	About 4 years
Cassia alata L. (Candle-bush, ringworm bush, golden candle stick)	Tropical America	Shrub	25	1 to 8 months
Cassia siamea Lam. (Iron-wood of East Indies, Kassod tree, Siamese senna)	East Indies	Tree	16	1 to 5 months
Caesalpinia pulcherrima (L.) Sw. (Barbados pride, Barbados flower fence)	Pantropic	Shrub	14	3 months
Caesalpinia sappan L. (Sappan wood, sapang)	Tropical Asia	Tree	4	1 to 4 months
Caesalpinia crista L. (Brazilletto, redwood, nickernut)	West Indies	Shrub	8	1 to 3 months
Caesalpinia sepiaria Roxb. (Wait-a-bit, Mysore thorn)	Tropical Asia	Shrub	10	1 to 6 months
Peltophorum inerme (Roxb.) Naves (Yellow poinciana)	Malasia	Tree	16	1 to 6 months
Mezoneurum kauaiense (Mann) Hillebr (Uhiuhi)	Hawaiian Islands	Tree	8	1 to 12 months
Delonix regia (Boj.) Raf. (Flamboyant, flame tree, royal poinciana)	Madagascar	Tree	23	1 to 12 months

survey warrant the assertion that these plants listed do not form nodules, but the facts do signify, on the other hand, that under these conditions of growth and habitat and by the methods of examination employed, nodules were not present.

DISCUSSION

Why certain leguminous plants bear nodules and others do not has provoked many attempts at explanation. Nobbe, et al. (21) were among the first to suggest that the physical character of the root hairs of several species in the sub-family Caesalpinioideae inhibited nodule formation. McDougall (19) expressed the same opinion as a result of his study on the thick-walled root hairs of species in the genus Gleditsia and related genera. Joshi (15) proposed the idea that root nodule bacteria might exist in the root tissues of certain leguminous plants, yet without root nodules being formed. A lower virulence of the specific bacteria or a slower reaction of the plant to the stimulus given by the bacteria was offered as an explanation for the lack of typical nodule formation. In 1926 Friesner (12) and Feher and Bokor (10) reported on the roots of *Gleditsia triacanthos* L. cylindrical swellings which contained bacteria and functioned as root nodules. Questionable evidence (15) has been offered showing that Rhizobia may exist in parts of the leguminous plant other than in nodules upon the roots, although a positive case was not found in *Cassia tora* L., when various histological procedures, involving combination fixing and staining methods were employed (1).

Whether the specific microörganism is present or not will continue to constitute the requisite proof with the majority of species to which lack of nodule formation is ascribed. As noted in table 1, *Cercis canadensis* L. was reported by Buckout (5), Harrison and Barlow (13), and others to be lacking nodules. In 1909 Buchanan (4) reported physiological studies on *Cercis* bacteria from nodules. Later reports tend to support the absence of nodules on this species. In 1889 Buckout (5) considered *Mimosa pudica* L. a non-nodulating plant. In 1932 Fred, Baldwin, and McCoy (11) reported that nodules sometimes occurred on roots of *Mimosa pudica* L. growing under greenhouse conditions. Later, in 1934, Leonard (18) reported the isolation of bacteria from the nodules of *Mimosa pudica* L. under conditions different from those commonly employed for other root nodule bacteria, and proposed the placing of this species in a separate cross-inoculation group. Recently Carroll (8) showed that nodule bacteria from a related species, *Mimosa strigillosa*, inoculated *Vigna sinensis* L. A somewhat similar case has been noted in the literature with respect to *Trifolium alexandrianum* L. In 1933 Sarkaria (22) reported this species as a non-nodulating plant under conditions of culture in India, although the species was placed in the clover group by Burrill and Hansen as early as 1917. Later the failure of the species to form nodules was explained by the fact that the root nodule bacteria were absent from the soil.

The outstanding fact remains, however, that some factor induces the penetration of the root nodule bacteria into leguminous root systems, and nodule formation results. Leonard (17) has epitomized the situation in the following manner: "As a matter of fact, the problem extends beyond the Leguminosae, for it has not been determined why, with few exceptions, bacteria root nodules

do not occur on non-leguminous plants, or, stated in another way, why they are peculiar to leguminous plants only."

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PHOSPHORUS DEFICIENCY IN CITRUS

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In order to add a so-called complete fertilizer, phosphorus is frequently added to soils in which citrus trees are grown. It is usually considered that, although not subject to appreciable loss from leaching, phosphorus is largely fixed in relatively unavailable forms and that the principal opportunity of the roots to absorb phosphorus occurs during a rather brief period subsequent to the time of application. Little importance appears to be attached to the continuous daily absorption from low concentrations of phosphorus obtained from so-called insoluble soil compounds or to the solubility and other physical or chemical soil effects that result from the use of organic manures and cover crops. Although these organic materials are rich in soluble phosphorus, only the nitrogen and organic matter ordinarily are given consideration, because they determine the cost. The relatively high phosphate concentration found in most citrus soils would appear to necessitate correspondingly large applications of nitrogenous fertilizers and may be involved in the relation of certain of the minor elements to healthy growth in citrus.

Because of the relatively low phosphorus requirement of citrus (the leaves and fruit contain considerably less phosphorus than either potassium or nitrogen) there is the greater uncertainty regarding the need of applying phosphorus to the soil. Indicator plants may be of value in detecting excesses or deficiencies of an element for trees, but the use of such plants is most limited, the best indicator plant being either the trees themselves or replants close by.

No case of proved phosphorus deficiency with citrus in the field has as yet been clearly demonstrated; in fact, the symptoms of phosphorus deficiency have not as yet been described. Interest rather has long been focused on the relation of excessive phosphorus absorption to the phenomenon of mottle-leaf.

This paper deals with the deficiency phase of phosphorus nutrition in citrus. It is shown that citrus trees can withstand a deficiency of phosphorus for a considerable time, apparently without injurious effects, and can absorb in a few days when phosphorus is relatively soluble, a supply that suffices for long periods.

METHODS

Plants in soil and sand cultures

Citrus plants were grown in Sierra loam soil outdoors in 12-gallon earthenware jars without drainage. Quartz sand (obtained from the Philadelphia Sand

Company) was used in cultures likewise conducted outdoors in 12-gallon glazed earthenware jars and also in galvanized iron cans 20 inches in diameter and 24 inches deep. Ottawa silica sand that was acid-treated and washed with distilled water was used in experiments in the glasshouse in 2-gallon glazed earthenware jars with drainage and in 6-liter capacity Swedish white enameled pans without drainage.

The following salts were used in preparing the culture solution: potassium nitrate, magnesium sulfate, sodium chloride, calcium nitrate, potassium dihydrogen phosphate, iron tartrate, boric acid, and manganese sulfate. The culture solution had the following composition (expressed as parts per million):

Na	K	Ca	Mg	NO_3	Cl	SO_4	PO_4	Fe	Mn	B	Total
7	185	159	54	718	10	216	105	1	0.1	0.1	1,455.2

Solution cultures

Solution culture experiments were conducted in Swedish white enameled pans of capacities varying from 4 to 18 liters and in pails of similar material but of 9-liter capacity. The culture solution was the same as that employed for the sand cultures and, unless otherwise stated, was not aerated nor was the pH controlled.

The citrus plants used in the soil, sand, or solution cultures consisted of budded trees or of rooted leafy-twig cuttings including various combinations of such cuttings as regards scion and stock.

Preparation and analysis of plant material

At the conclusion of an experiment the plants were photographed and then subdivided into the various portions, such as leaves, twigs, and bark. The samples were rapidly washed with distilled water, wiped clean, and dried in a large well-ventilated oven at 65°C. The materials were later ground either by hand in a tinned mill or by means of a motor-driven Wiley mill, after which the samples were redried.

Chemical analysis

The methods employed for the determination of the various constituents were those used by Haas and Bliss (8). Phosphorus in the dry matter was determined by the ashing method employed by Howk and DeTurk (11) followed by the molybdenum blue colorimetric method essentially as used by Truog and Meyer (15). The various phosphorus fractions in the dry matter were determined by the method of DeTurk, Holbert, and Howk (2).

EXPERIMENTAL

The chief objective in the culture studies was to grow citrus in sand and solution cultures in which phosphate was omitted and to note the effect, not only on the appearance of the trees, but also upon their chemical composition.

Haas (6) has reported the effect of various concentrations of phosphate on the growth and composition of lemon cuttings produced in culture solutions.

The solution cultures on phosphorus deficiency, to be reported later in this paper, were a valuable supplement to the sand cultures because of the more rigid control possible in the glasshouse. Although the sand cultures grown outdoors were well covered, they were nevertheless the recipients of phosphorus-containing dusts, which, if they do not reach the sand immediately, eventually are washed there by the rains.

TABLE 1

Phosphorus content of various portions of citrus trees

TREES	PHOSPHORUS, AS PER CENT OF DRY MATTER, IN				
	Leaves	Shoots	Bark of trunk	Bark of roots	Young roots
Lisbon lemon cuttings grown 2 years in phosphate-deficient sand cultures	0 0688	0 0300	0 0388	0 0538	0 0694
Lisbon lemon tree on sweet-orange stock; grown 2 years in soil in nursery	0 4125	0.3063	0.0575	0 0850	0.1450
Valencia orange trees on sweet-orange stock; grown 2 years in phosphate-deficient sand cultures	0 0550	0 0425	0 0400	0 0463	0.0625
Valencia orange tree on sweet-orange stock; grown 2 years in soil in nursery	0 6500	0 2750	0 0875	0 0825	0 2000

	Bark of 1928 shoots	Bark of 1927 shoots	Bark of 1928 trunk elongation	Remainder of trunk		Bark of main tap roots	Bark of side roots
				Bark of upper half	Bark of lower half		
Rough lemon seedlings 3 years old from seed; grown in soil in nursery until October, 1928	0 0825	0 0800	0.1200	0 1100	0.0925	0 1175	0 1063

Trees grown in phosphate-deficient sand cultures and in soil

Three Lisbon lemon cuttings and three Valencia orange trees budded on sweet orange as rootstocks were transplanted bare root (washed with tap and then with distilled water) from soil in the nursery to sand cultures in galvanized iron cans. The trees were grown in these sand cultures for approximately 2 years, during which time the cultures received a complete nutrient solution so modified as to substitute nitrate for phosphate. At the conclusion of the experiment the trees had a basal trunk caliper of approximately 1 to 1½ inches, and showed symptoms typical of phosphate deficiency.

The percentages of phosphorus in the dry matter of the various portions of these young trees are given in table 1. The leaves of the trees in the phosphate-deficient sand cultures contain approximately 0.05 to 0.07 per cent of phosphorus in the dry matter; the leaves of trees in soil contained many times

this amount. The soil was markedly deficient in nitrogen, and, as Haas (7) has shown, the leaves of trees in such soils contain higher percentages of phosphorus. The shoots and the trunk and root bark of the phosphate-deficient cultures contained less phosphorus than did the leaves or young roots.

One of the lemon trees in the phosphate-deficient cultures produced several lemons. These fruits contained 0.06 per cent of phosphorus in the dry matter, approximately that found in the leaves and roots.

A group of Rough lemon seedling trees 3 years of age from seed were obtained in the nursery and were used for the determination of the percentage of phosphorus in the bark of young trees. The bark of the shoots (table 1) contained smaller percentages of phosphorus than that of the trunk. A gradient in the

TABLE 2

Phosphorus fractions of Valencia orange and Lisbon lemon trees grown in phosphorus-deficient sand cultures and in soil

VARIETY	PORTION USED	PHOSPHORUS AS PER CENT OF DRY MATTER					
		Inorganic		Organic		Phospholipid	
		Phosphorus-deficient sand cultures	Soil	Phosphorus-deficient sand cultures	Soil	Phosphorus-deficient sand cultures	Soil
Valencia orange	Mature leaves	0 0210	0 4625	0 0195	0 2900	0 0065	0 0188
	Mature shoots		0 1550		0 0825		0 0067
	Bark of trunk	0 0120	0 0600	Trace	0 0330	0 0034	0 0052
	Bark of main root	0 0138	0 0725	Trace	0 0183	0 0053	0 0060
	Young rootlets	0 0188	0 1358	0 0145	0 0359	0 0036	0 0088
Lisbon lemon	Mature leaves	0 0300	0 3750	0 0112	0 1600	0 0053	0 0215
	Mature shoots	0 0133	0 1900	Trace	0 0750	0 0022	0 0101
	Bark of trunk	0 0100	0 0575	Trace	0 0250	0 0047	0 0057
	Bark of main root	0 0133		Trace		0 0042	
	Young rootlets	0 0200	0.0950	0 0110	0 0370	0 0034	0 0074

phosphorus content occurred in the trunk bark, with the lowest percentage near the base of the trunk.

The shoots and bark of citrus trees grown in phosphorus-deficient sand cultures are the first portions in which the percentages of organic phosphorus are reduced to a trace. The data in table 2 suggest that organic phosphorus within the tissues may be used by citrus when conditions for obtaining inorganic phosphorus are desperate.

The percentages of inorganic and organic phosphorus shown in table 2 decrease from the leaves to the shoots and trunk and increase from the main root to the young rootlets. The percentages of the various phosphorus fractions are generally highest in the leaves, even after a prolonged period of phosphorus starvation.

In table 3 are presented the percentages of inorganic, organic, and phospholipid phosphorus in leaves from trees grown in artificial solution cultures. When phosphate was present for only a few days at intervals of several weeks, the percentage of inorganic phosphorus in the leaves of Valencia orange leafy-twig cuttings was 0.1293, with no accompanying symptoms of phosphorus deficiency. In the absence of phosphorus in the culture solution, symptoms were developed to a marked degree, and the percentage of inorganic phosphorus in the dry matter of Valencia orange leaves was 0.0175 and in Eureka lemon leaves 0.0388. Drastic reductions in the percentages of organic and phospholipid phosphorus resulted when phosphorus was omitted from the culture solution.

TABLE 3

Phosphorus fractions in citrus leaves from trees grown with deficient phosphate in artificial cultures

CULTURES	VARIETY	PHOSPHATE CONCENTRATION IN THE CULTURE MEDIUM	PHOSPHORUS AS PER CENT OF DRY MATTER		
			Inorganic	Organic	Phospholipid
Valencia orange cuttings as scions on Rough lemon cuttings as stocks; grown from March 2, 1933, to June 18, 1934	Valencia orange	Solution cultures (control); 105 p.p.m. PO_4 for a few days at intervals of several weeks	0.1293	0.0550	0.0084
Valencia orange cuttings as scions on Eureka lemon cuttings as stocks; grown from March 2, 1933, to June 18, 1934	Valencia orange	Solution cultures; no PO_4	0.0175	0.0088	0.0028
Eureka lemon cuttings; grown from April 11 to December 26, 1933; cultures maintained at pH 4.5 with HNO_3	Eureka lemon	Solution cultures; no PO_4	0.0388	0.0078	0.0038

The Eureka lemon roots of the grafted cuttings in which Valencia orange leafy-twig cuttings were used as the scions (table 1) contained 0.0100, 0.0108, and 0.0035 per cent, respectively, of inorganic, organic, and phospholipid phosphorus in the dry matter. These results approximate those found for the Valencia orange leaves of the same trees (table 3) and point to an equilibrium in the phosphorus distribution of such phosphate-starved plants. This same condition is also shown in the percentages of organic phosphorus in the leaves and roots of phosphorus-deficient sand cultures with lemon trees (table 2).

In view of the results of Heck and Whiting (10) and others, who found that organic phosphorus was utilized by certain plants, and of the results of Pierre and Parker (14), who found that organic phosphorus was not available for plant growth, it was considered desirable to ascertain whether or not lemon cuttings could utilize organic phosphorus.

Many experiments were carried on with lemon cuttings grown in nonaerated culture solutions containing no phosphate, but to which phytin was added. The cuttings in most cases showed a growth response. Agitation of the solution at intervals, by means of a stirring rod, to insure contact of the roots with the phosphorus, greatly improved the growth.

In experiments with aerated cultures, lemon cuttings were grown without phosphate until every mature leaf had fallen and only a single long shoot with phosphorus-deficient leaves at the tip remained. Phytin was then added to the solution of such cultures in 6-liter pans. Within a few days, many of the lateral buds and many of the roots began to grow. Phytin appears to be of value as a source of phosphorus for citrus when the phosphorus supply is deficient.

Symptoms of phosphorus deficiency in leaves

One phase of the symptoms of phosphorus deficiency in Valencia orange leaves is shown in plate 1, figure 1, in which the chlororphyll is seen to fade in certain areas. Another phase is indicated in plate 1, figure 2, by the burning of the blade at the tip, which without careful diagnosis may be confused with injury caused by high chlorine; by the burning of the blade in various locations other than at the tip, as shown in the leaf on the right in the upper row; by the reduction in leaf size; and by the dull brownish green color and lack of luster.

In plate 1, figure 3, are seen some of the symptoms typical of phosphorus deficiency in lemon leaves taken from cuttings grown in solution cultures in the glasshouse. There is no uniformity in the location of the leaf-burn, and abscission soon follows the burning. The subsequent new leaves, when mature, were dwarfed, brittle, and burned, and possessed a characteristic dull brownish green color. A deficiency as indicated by such symptoms was evident in the leaves of all Lisbon lemon cuttings grown in culture solutions containing up to and including 1 p.p.m. of phosphate. At this highest concentration the symptoms were evident in a minor way only in a few leaves. The roots of phosphate-deficient cuttings also were affected and will be described later.

With the phosphate-deficient Valencia orange and Lisbon lemon sand cultures grown outdoors were similar cultures in which walnut trees (*Juglans regia*) were grown. Likewise, in aerated solution cultures in the glasshouse, walnut seedlings were grown without phosphate. No symptoms of any deficiences produced under controlled conditions have as yet been described for walnut trees. Plate 1, figure 4, shows the burning or death of tissue as a result of a phosphate deficiency in sand cultures. In the solution cultures the burning frequently was very severe. Walnut trees utilize large amounts of phosphorus, and these preliminary results suggest the similarity of the effects of a phosphate deficiency on leaves of citrus and walnut trees.

In the glasshouse at first, Swedish enamel ware pans were used for sand cultures without drainage. The Ottawa silica sand was acid-treated and

washed with distilled water prior to being used. Solutions were renewed by periodically flooding the sand with either distilled water or culture solution or with both, followed by decantation of the excess solution.

The growth of Lisbon lemon cuttings in the three cultures lacking phosphate far exceeded that in the other cultures, as is shown in plate 1, figure 5. This experiment proved to be a starting point in an investigation regarding the concentration of phosphorus in relation to growth.

In a second experiment with Lisbon lemon and Valencia orange cuttings, the (Philadelphia) silica sand was acid-treated and washed with distilled water. Some cultures received a complete solution containing 105 p.p.m. of phosphate, while others received a solution lacking phosphate. The omission of phosphorus was accompanied by an increased growth of the tops, but after a time the leaves of the cultures receiving no phosphorus showed burning typical of phosphate deficiency.

The leaves of the control cultures had to be wiped free of spiders every little while, although the spiders failed to become established on the leaves of adjacent phosphate-deficient cultures. Phosphate-deficient leaves are more brittle, and perhaps the leaf surfaces are more difficult to penetrate. No trouble at any time from scale insects was encountered in these cultures lacking phosphate. Haas (5) has shown that citrus scale insects on fruits contain relatively large amounts of phosphorus.

In plate 2 are shown transverse sections of Lisbon lemon leaves obtained from cuttings grown in a complete culture solution and in such a solution lacking phosphate. The cells in the palisade tissue are narrow and elongated (pl. 2, fig. 1) when phosphorus is abundant and are nearly square (pl. 2, fig. 2) when phosphate is deficient in the culture solution. The cells of the phosphorus-deficient section reveal very few contents as compared with those of the control section. This condition, together with changes in cell structure and toughness or brittleness of the leaves, may be a factor inhibiting the action of the mite commonly called "red spider." A decreased rate of cell division, an increased cell wall thickening, and the occurrence of cortical cells similar in shape to these palisade cells have been described as occurring in phosphate-deficient tomato plants by Kraybill (12) and by Eckerson (3).

The early symptoms of phosphorus deficiency in lemon cuttings are seen in plate 3, figure 1. There is an increased shoot growth, which is one of terminal elongation accompanied by an almost total absence of lateral shoots. The leaves absciss from the lowermost portions of the new shoots produced by the original cuttings. The leaves of the original cuttings remain attached long after the symptoms of deficiency appear in the young leaves. The younger leaves usually are smaller than the older ones, although there is no gradation of leaf size from the base of a shoot to the tip. Frequently a series of small leaves may be followed in the next growth cycle by fewer but considerably larger leaves. Leaves at the stage shown in plate 3, figure 1 *B*, begin to show burned areas as previously described, and although the leaves feel brittle and

stiff, many of them droop at the petiole in a direction parallel to the shoot axis.

The symptoms of phosphate deficiency of lemon cuttings grown in non-aerated cultures and the recovery therefrom will next be considered in connection with advanced stages of phosphorus deficiency. Plate 3, figure 2 *A* shows an extreme case of such deficiency in lemon cuttings grown in a culture solution lacking phosphate and aluminum. The lower or mature leaves have fallen, the roots have become dark in color and have ceased growing, and the lateral bud growth has remained suppressed.

It is seen in plate 3, figure 2 *B*, that the lateral buds are alive and able to grow once phosphorus becomes available. The cuttings in *B* were grown without phosphorus or aluminum in the culture solution until the growth resembled that shown in *A*. Then 105 p.p.m. of phosphate but no aluminum was used in the culture solution for a few days at intervals of several weeks. Note the recovery in *B* that resulted from the use of phosphorus after a long period of phosphorus starvation. The pH of the solution was not controlled and at times ranged between 7 and 8.

The first effects of the addition of phosphate were seen in the tops. Within a week or so after phosphate was used in the culture solution, the new terminal growth ceased to consist of small, dull, brownish green leaves. The new leaves were large and healthy, and abscission ceased for the time being. The old roots were short, brown, and stubby, but otherwise in good condition, and ultimately began to grow. Very few, if any, of the lateral buds started growth until sometime later.

The retained leaves apparently first manufactured food to supply their own needs and then secondarily those of the roots and lateral buds. The new roots did not appear until months after the tops had shown the recovery evident in plate 3, figure 2 *B*. The delayed root recovery suggests that, in the absence of aluminum to precipitate or retain phosphorus in or on the root tissue during phosphate starvation and early recovery, a marked depletion of phosphorus and carbohydrate reserves possibly may take place in the roots. Haas (6) has shown the relation of the sugar content of lemon leaves to the phosphate concentration in the culture solution.

When phosphate-deficiency symptoms occur in lemon cuttings grown without phosphate in the culture solution, the phosphorus contents of the dry matter of the leaves fall in the range from 0.0363 to 0.0736 per cent. The varied locations of burn in phosphate-deficient leaves are indicative of considerable variation in the phosphorus distribution in leaves or of the degree of sensitivity of different tissues to phosphate deficiency.

Symptoms of deficiency were overcome in part only by spraying phosphorus-deficient Valencia orange trees grown in sand cultures and lemon cuttings grown in solution cultures, with a mixture containing phosphate. For this purpose a solution was used consisting of 10 gm. of $CaH_4(PO_4)_2$, 5 gm. of $Ca(OH)_2$, and 100 cc. of water containing blood albumin spreader. No benefit resulted when the $Ca(OH)_2$ was omitted.

Heretofore, the discussion has been confined largely to the symptoms of phosphorus deficiency in lemon trees. Attention is now directed toward such a deficiency or an excess in relation to the growth of Valencia orange and pomelo cuttings. The absence of data regarding the phosphate nutrition of orange and grapefruit cuttings has greatly retarded the successful growing of such trees in culture solutions. With 105 p.p.m. of phosphate in the non-aerated or aerated culture solution (pl. 4, fig. 1) the cuttings start growth, but after the first new cycle the original leaves gradually fall off, and the growth of the roots comes to a standstill.

Many orange and grapefruit cuttings were discarded because of their failure to grow. By experimentation it was found that the use of 105 p.p.m. of phosphate for a few days just prior to the renewal of the culture solution at intervals of several weeks and, as shown elsewhere, the use of 5 to 10 p.p.m. of aluminum as citrate when phosphate was absent permitted healthy, vigorous growth.

In plate 4, figure 2, are seen Valencia orange cuttings as scions on Rough lemon cuttings as stocks after being grown in a phosphate-free culture solution containing no aluminum. The growth of the roots came to a standstill while terminal growth was still being made by the shoots. The leaves were of a dull brownish green color and many of the lowermost ones were falling.

The cuttings illustrated in plate 4, figure 3, show a striking contrast in growth when phosphate was deficient. The continued use of 105 p.p.m. of phosphate produced a healthy top but a most abbreviated root system (pl. 4, fig. 3 *A*). When, however, phosphate was omitted from the culture solution (pl. 4, fig. 3 *B*) the growth of the roots showed improvement for a time, but gradually the oldest leaves were lost until the tops of the cuttings presented a thin appearance.

The growth of pomelo leafy-twig cuttings was improved (compare with plate 4, figure 1 *B*) by using 105 p.p.m. of phosphate in the culture solution for only a few days at intervals of several weeks, as shown in plate 5, figure 1. Note the coarse, heavy type of root characteristic of pomelo cuttings. Additional improvement in growth was later brought about by the use of 5 to 10 p.p.m. of aluminum in the culture solution during the interval of several weeks when phosphate was omitted.

The effect of advanced stages of phosphate deficiency in pomelo cuttings will next be considered. In plate 5, figure 2, are seen pomelo cuttings as scions on Eureka lemon cuttings as stocks. In *A*, the original leaves still remain attached, while most of the oldest new leaves have fallen and there is a suppression of growth of the lateral buds. This inhibition of the growth of lateral buds suggests a possible relation of phosphorus to the delayed foliation of deciduous trees. In *B*, all of the leaves have fallen except the very youngest. The roots are coarse and, although their growth is at a standstill, in neither case have they broken down.

The leaves are a dull brownish green color. The roots (in plate 5, figure 2 *A*, and in many other cuttings among these cultures) consisted of thick, coarse

laterals with few fine roots. Many of these lemon cuttings used as stocks have produced roots of the coarse type usually found on rooted pomelo cuttings. This may be due in part to the active vegetative growth of the pomelo variety and the availability of large supplies of organic materials because of the extensive leafy growth. This effect of scion on stock also has been referred to by Halma (9), who found that the Eureka lemon scion, when grafted to twigs of sour orange, effected a change in the inherent form of the root system of the latter.

TABLE 4

Phosphorus content of various portions of young Valencia orange and pomelo trees grown in solution cultures deficient in phosphorus

VARIETY	DURATION OF THE EXPERIMENT	CULTURE SOLUTION	PHOSPHORUS, AS PER CENT OF DRY MATTER, IN		
			Leaves	Shoots	Roots
Valencia orange cuttings as scions on Eureka lemon cuttings as stocks	March 2, 1933, to June 18, 1934	PO_4 absent	0 0419	0 0306	0 0463
Valencia orange cuttings as scions on Lisbon lemon cuttings as stocks	June 7, 1933, to June 18, 1934	PO_4 absent	0 0563	0 0313	0 0500
	June 7, 1933, to June 20, 1934	PO_4 absent	0 0425*	0 0256	0 0525
Valencia orange cuttings as scions on Rough lemon cuttings as stocks	June 7, 1933, to May 25, 1934	PO_4 absent	0 0556	0 0325	0 0438
	June 7, 1933, to June 20, 1934	105 p.p.m. PO_4 present	0 1500	0 2150	..
Pomelo cuttings on Eureka lemon cuttings as stocks	March 13, 1933, to June 18, 1934	PO_4 absent	0 0469	0 0288	0 0500
	August 29, 1931, to June 19, 1934	PO_4 absent	0 0425	0 0338	0 0475

* Phosphorus deficiency very advanced; considerable leaf abscission.

Table 4 presents data in regard to the percentage of phosphorus in the various portions of Valencia orange and pomelo cuttings grown in phosphate-deficient culture solutions. When the phosphate deficiency symptoms were obvious, the percentages of phosphorus for the leaves and roots were approximately equal and those for the shoots were intermediate. This phosphorus equilibrium in leaves and roots may indicate a standstill condition of growth. When 105 p.p.m. of phosphate was used in the culture solution the percentage of phosphorus in the shoots exceeded considerably that in the leaves.

Lemon cuttings grown in a nonaerated culture solution containing 105 p.p.m.

of phosphate made fair growth for a time, after which the growth of the root system came to a standstill, as shown in plate 6, figure 1 *A*. The leaves frequently were chlorotic, notwithstanding the liberal use of ferric tartrate in the culture solution. When this decline was evident, traces of many elements not ordinarily used in culture solutions (4) were added but without benefit. This suggested that either the phosphate was preventing the traces of elements from functioning or that the phosphate itself was excessive and a factor in bringing about injury to the roots of nonaerated cultures.

The culture solution used for the growth of the cuttings (pl. 6, fig. 1 *A*) was subsequently modified by omitting the phosphate and adding the minor elements. The removal of phosphate was accompanied by a marked improvement in the growth of the roots and tops, as shown in *B*.

It is still possible that the actual decrease in the phosphate concentration in the culture solution was not solely responsible for the improvement in the growth. The removal of phosphate permits the traces of elements to remain better in solution, and these may then have brought about the improvement. Results thus far obtained have shown a beneficial effect of a temporary depletion of the phosphate supply and the further benefit of aluminum during such phosphate depletion in unaerated culture solutions. Haas (6) has described the excellent growth obtainable when 105 p.p.m. of phosphate is used in a thoroughly aerated culture solution. The results indicate a possible relation of aeration with phosphate absorption and health in roots. Aeration apparently becomes of greater importance as the concentration of phosphate in the culture solution increases.

Phosphorus deficiency in lemon cuttings in relation to silica

Many investigators (1, 13, and others) have shown the interrelation between silica and phosphorus in plants and are in agreement that silica may partially replace phosphorus. This relation of silica to phosphorus was tested in preliminary experiments by growing lemon cuttings in a culture solution lacking phosphorus and then adding silicic acid to the solutions in which a number of the cuttings were grown.

Plate 6, figure 2, shows the differences in growth produced by the addition of silicic acid to culture solutions lacking phosphorus. Other Lisbon lemon cultures, grown in a similar manner but for a short period (February 18 to March 22, 1935), showed the better growth in the presence of silicic acid when phosphate was absent. This growth response was independent of the changes in pH brought about by the addition of the silicic acid to the culture solution because the pH of all cultures in these experiments was maintained at approximately the same favorable values, 6 to 6.5.

SUMMARY

A study was made of the deficiency phase of phosphorus nutrition in citrus by the use of soil, sand, and solution cultures. The effect of such a deficiency

was noted on the appearance of the trees and also on their chemical composition.

The leaves of trees in phosphate-deficient sand cultures contained approximately 0.05 to 0.07 per cent of phosphorus in the dry matter, whereas the leaves of trees in soil (low in nitrate nitrogen) contained several times these percentages. The shoots and the trunk and root bark of the phosphate-deficient cultures contained smaller percentages of phosphorus than the leaves or young roots. Lemon fruits of phosphate-deficient cultures contained 0.06 per cent of phosphorus in the dry matter, a value approximating that found in the leaves and roots.

The shoots and the bark are the first portions to have the percentages of their organic phosphorus fraction reduced to a trace when phosphorus is omitted from the culture solution. Organic phosphorus within the tissues or in the culture solution may be used by citrus when conditions for obtaining inorganic phosphorus become desperate. The percentages of phospholipid phosphorus are highest in the leaves even after a prolonged period of phosphorus starvation.

Symptoms of phosphorus deficiency in citrus leaves are variable and may consist of a fading of the chlorophyll, a burning of the blade in various locations, a reduction in leaf size, or a change to a dull brownish green color without luster.

A phosphorus deficiency brings about changes in the shape and contents of the cells of lemon leaves. There is an increased shoot growth, but it is merely one of terminal elongation accompanied by an almost total absence of lateral shoots. In severe cases the leaves not only burn but feel brittle; they droop at the petiole in a direction parallel to the shoot axis and later absciss. Inhibition of the growth of lateral buds can be overcome by the addition of phosphate to the culture solution.

The growth of Valencia orange and pomelo leafy-twig cuttings was improved by using 105 p.p.m. of phosphate in the unaerated culture solution for only a few days at intervals of several weeks. This method of supplying phosphorus was superior to the continuous presence of lower but still somewhat high concentrations of this element.

In the absence of phosphorus in the culture solution, silica appeared to be of benefit.

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PLATE 1

Fig. 1. Valencia orange leaves from trees grown in sand cultures outdoors in large galvanized iron containers 20 inches in diameter and 24 inches deep. Phosphate was omitted in the culture solution and resulted in the loss of chlorophyll.

Fig. 2. Valencia orange leaves from trees in sand cultures that received a culture solution lacking phosphate. The leaves in the upper row show the reduction in leaf size and the tip and side burn found on leaves of trees grown in galvanized iron containers 20 inches in diameter and 24 inches deep. The lower row of leaves was obtained from trees in sand cultures in 12-gallon earthenware pots. The two leaves on the left in the lower row show gum formation on the under side. The leaves in both rows have a dull brownish green color and a lack of luster.

Fig. 3. Leaves of lemon cuttings as affected by phosphorus in solution cultures: Lower left, healthy dark green leaf from a cutting grown in a solution containing 105 p.p.m. PO_4; remaining leaves from cuttings grown in solutions containing no phosphate. Note the nonuniformity of the location of the leaf burn.

Fig. 4. Dead areas in leaves of walnut trees (*Juglans regia*) grown in sand cultures that received a culture solution lacking phosphate. This condition was frequently found along the veins as shown in the two leaves on the left, although scattered dead areas also were found, as shown in the two leaves on the right. There is no uniformity in the location of the burned areas. In severe cases the burn may cover the entire leaf.

Fig. 5. Lisbon lemon leafy-twig cuttings in preliminary sand cultures. Left to right: complete culture solution (control); sodium substituted for potassium; magnesium for potassium; calcium for potassium; phosphate omitted, and 10 p.p.m. aluminum added as citrate; phosphate omitted, and 20 p.p.m. aluminum added as citrate; and phosphate omitted. The increased growth of the three phosphate-free cultures over that of the control is clearly indicated.

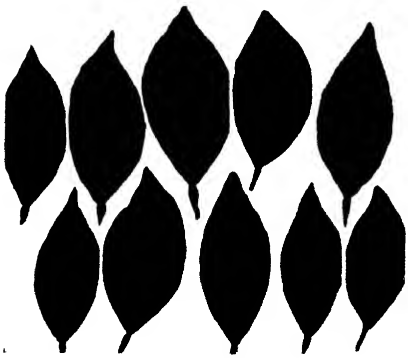

FIG. 1

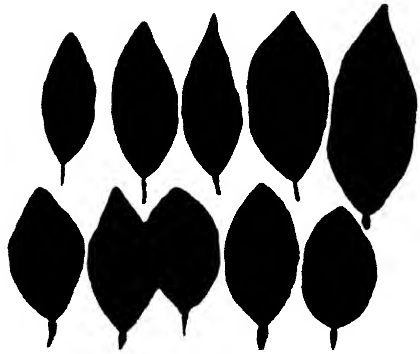

FIG. 2

FIG. 3

FIG. 4

FIG. 5

PLATE 2

TRANSVERSE SECTIONS OF LISBON LEMON LEAVES FROM LEAFY-TWIG CUTTINGS GROWN IN THE GLASSHOUSE

FIG. 1 Leaves from cuttings grown in a complete culture solution

FIG. 2 Leaves from cuttings grown in a complete culture solution except that phosphate is omitted

FIG. 1

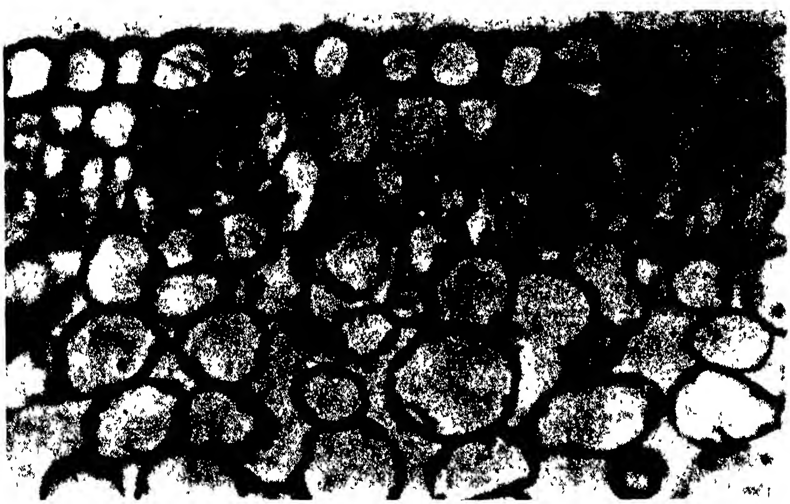

FIG. 2

PLATE 3

FIG. 1 Early symptoms of phosphate-deficiency in lemon cuttings *A*, cuttings grown in a culture solution containing phosphate The internodes are short and the leaves of good size *B*, cuttings grown in a culture solution without phosphate The shoots generally remain unbranched and have, therefore, only a terminal growth, the youngest leaves are of reduced size, and the oldest leaves produced while in culture solution absciss after drooping at the petiole for some time in a direction parallel to the shoot axis

FIG. 2 Recovery of Lisbon lemon leafy-twig cuttings from phosphate deficiency *A* effect of growing cuttings from July 14, 1932, to November 9, 1933, in a culture solution lacking phosphate and in which the pH is not controlled *B*, the state of recovery on November 9, 1933, of cuttings grown from February 19 to June 7, 1933, in a culture solution containing no phosphate or aluminum and grown from June 7 to November 9, 1933, in culture solution in which phosphate (105 p p m) was present only for a few days at intervals of several weeks

A. R. C. HAAS

FIG. 1

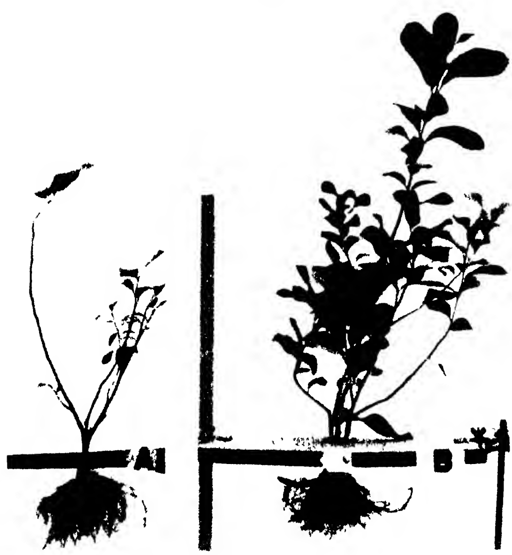

FIG. 2

PLATE 4

FIG. 1 *A*, Valencia orange leafy-twig cuttings, *B*, pomelo leafy-twig cuttings These were grown in a culture solution containing 105 p p m PO_4 beginning on January 8, 1933 (photo May 11, 1934) Most, if not all, of the original leaves on the cuttings have fallen, and growth has come to a standstill This condition was corrected by the judicious use of phosphate and aluminum

FIG 2 Valencia orange leafy-twig cuttings as scions on Rough lemon leafy twig cuttings as stocks. The cuttings were grown from June 7, 1933, to May 25, 1934, in a culture solution in which phosphate was omitted Note the loss of the oldest leaves and the exudation of gum from leaf scars Roots were healthy, although under the conditions they were unable to continue the growth of young rootlets

FIG. 3 Valencia orange leafy-twig cutting as the scion on Rough lemon leafy twig cutting as the stock. *A*, grown in nonaerated culture solution (containing 105 p p m PO_4 and five times the normal concentration of calcium nitrate) beginning on June 7, 1933 (photo September 13, 1933) Note the retention of leaves but the dwarfed root system. *B*, grown in nonaerated culture solution (containing no phosphate but containing five times the normal concentration of calcium nitrate) beginning June 7, 1933 (photo September 13, 1933) Note the more sparsely foliated tops and more elongated root system when phosphate was deficient (*B*)

FIG. 1

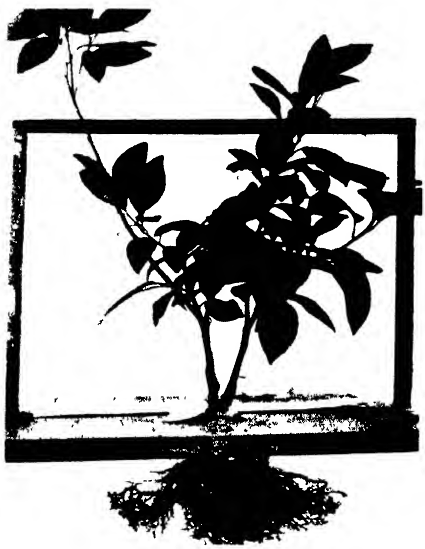

FIG. 2

FIG. 3

PLATE 5

FIG. 1. Pomelo leafy-twig cuttings grown in a culture solution containing 105 p p m PO_4. The cuttings grew poorly until the phosphate was allowed to be present for only a few days at intervals of several weeks.

FIG. 2. Advanced stages of phosphorus-deficiency in pomelo leafy twig cuttings as scions on Eureka lemon leafy-twig cuttings as stocks. The cuttings were grown in a culture solution in which phosphate was omitted. *A*, cuttings grown from August 29, 1931, to December 26, 1933, show the loss of the oldest leaves on the new shoots while the original pomelo leaves still remain attached; *B*, cuttings grown from January 8, 1931, to May 11, 1934, show the loss of all except the youngest new leaves. Root growth is at a standstill. Note the absence of any growth of buds in the axils of leaves that have fallen.

FIG. 1

FIG. 2

PLATE 6

FIG. 1 Effect of phosphate on the root growth of lemon leafy-twig cuttings grown from July 16, 1932, until September 13, 1933, in nonaerated culture solution containing 105 p p m. phosphate *A*, ineffectiveness of adding to the culture solution 0 1 p p m of the minor elements (4) including zinc and copper; *B*, response following the omission of the phosphate and the addition of 0 1 p p m. of the minor elements, as in *A*. The improvement in non-aerated cultures following the omission of phosphate without the addition of the minor elements other than iron, boron, and manganese has been repeatedly shown in other experiments.

FIG. 2 Effect of silica in the absence of phosphate on the growth of Lisbon lemon leafy-twig cuttings The cuttings were grown in culture solutions from January 7 to March 22, 1935 Left, two cultures from solutions containing 0 1 gm silicic acid (H_2SiO_4) in 6 liters, right, two cultures from solutions containing no added silica (control).

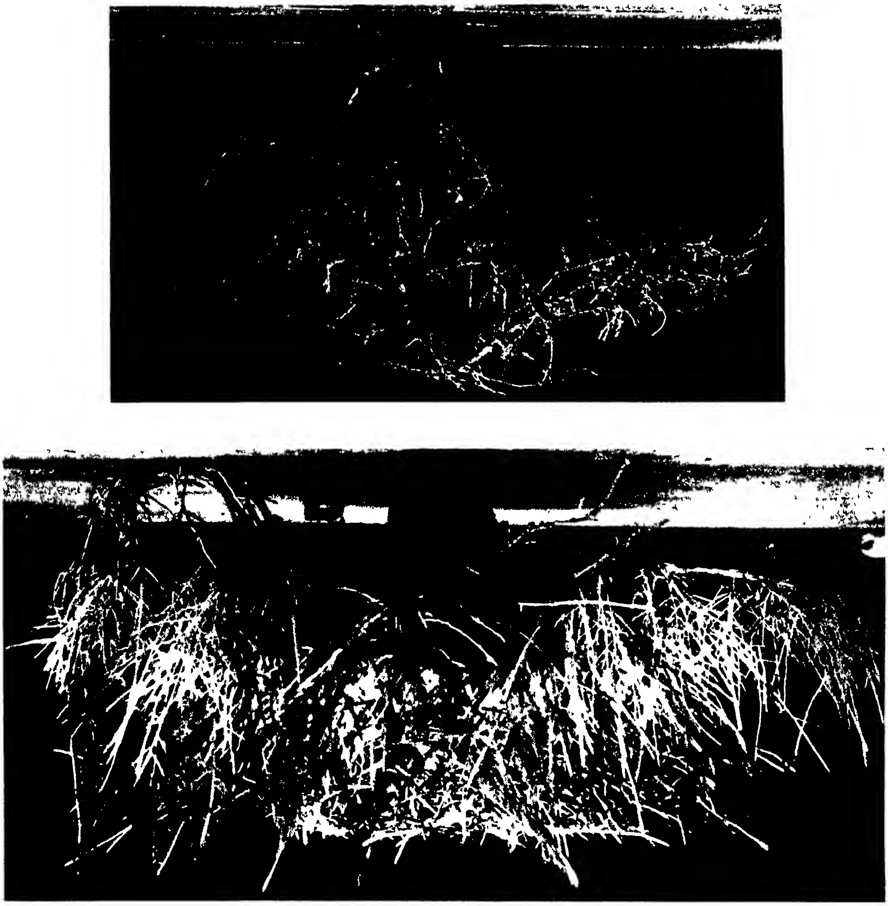

Fig. 1

Fig. 2

DECOMPOSITION OF LIGNIN BY MICROÖRGANISMS[1]

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The problem of lignin transformation in nature by chemical and biological agencies and the rôle of this process in the formation of humus in soils and in composts, as well as of peat and of coal, have attracted, during the last two decades, considerable attention from chemists, biologists, and agriculturists. The results obtained so far, however, are hardly sufficient to justify a clear conception of the mechanism of lignin decomposition and of the agents concerned in this process.

The accumulated evidence concerning the action of microorganisms upon lignin, in the process of decomposition of plant and animal residues in nature, can be summarized as follows: 1. Lignin is, among chemical complexes, most resistant to attack by fungi, bacteria, and invertebrate animals living in soils, peat bogs, and composts (6, 12, 15, 18). 2. Lignin does not accumulate quantitatively in the same state in which it exists in plant materials but undergoes a slow process of transformation (1, 2, 3, 14). 3. Certain specific groups of microörganisms, found largely among the higher fungi, are capable of bringing about active destruction of lignin, frequently even to a greater extent than that of cellulose and hemicelluloses (4, 5, 21). 4. Lignin is subject to decomposition by these organisms as long as it is present in the fresh or in the partly decomposed plant tissues; once it is prepared in a purified state, it becomes completely resistant to attack even by these organisms. It still remains to be determined whether this is due to the physical or the chemical modification of the lignin complex in the process of its preparation. Lignin produced biologically, as in the decomposition of wood by cellulose-destroying fungi, whereby it is left in a free state, is also resistant to the action of lignin-decomposing microörganisms (3, 11, 17). 5. The effect of lignin upon microbial activities, such as cellulose decomposition, glucose fermentation, and nitrate formation, is not injurious and may even be favorable (19). 6. The chemical nature of lignin in a growing plant varies with the age of the plant,

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[2] The authors are indebted to Prof. W. Fuchs, for suggesting the methods of lignin preparation and determination and for collaborating in the chemical part of the investigations; also to Mr. T. C. Cordon, for assisting in the preparation of the alkali lignin and in various chemical analyses.

as shown by an increase in the methoxyl content of the lignin with an increase in the maturity of the plant. This seems to be correlated with the increase in the resistance of the lignin to decomposition, as shown by the fact that the younger the plant the more readily the different plant constituents, including the lignin, undergo decomposition (20). Whether a similar correlation exists between the chemical nature of the lignins in different plants and the rapidity of their decomposition by microörganisms still remains to be determined. 7. Because of its greater resistance to decomposition than is the case of the other plant constituents, the concentration of lignin in the residual material increases with the advance of decomposition of plant residues in soils, composts, and peat bogs (8, 12). Since the decomposed material is usually designated as "humus," the formation and accumulation of humus may be recognized as depending upon lignin accumulation, although lignin may not be the only organic complex contributing to humus formation. 8. The gradual transformation of the lignin noted above consists in (*a*) a loss of methoxyl groups, (*b*) darkening in color, accompanied by absorption of oxygen, (*c*) combination with proteins, (*d*) increase in alkali solubility. As a result of these changes, the alkali-soluble fractions of the humus, commonly referred to as "humic acids," partake of the properties of lignin as modified above, these modifications being characterized largely by an increase in nitrogen content, a lower methoxyl content, and a darker color (7, 13, 14).

In order to study the modifications and changes produced in the lignin molecule by microörganisms capable either of destroying it completely or of modifying only certain parts of it, one must be able to grow these organisms upon isolated lignin. The fact that all previous attempts to grow fungi, which have been found capable of decomposing lignin in natural plant materials or in composted plant residues, upon isolated lignin failed, is believed to be a result of the changes produced in the lignin molecule in the process of its isolation by drastic treatments. Usually either acid lignin or alkali lignin has been employed. When lignin is spread upon a silica gel plate and inoculated with a soil suspension, an abundant population of bacteria, fungi, actinomyces, and invertebrate animals is usually found to develop. However, when these organisms are isolated in pure culture and tested upon the same lignin in agar or liquid media, either they fail completely to attack the lignin or their action is so slow and so limited as to make accurate determinations valueless. An attempt has, therefore, been made to utilize another form of lignin, namely, phenol-lignin (9), as a substrate for the growth of lignin-decomposing organisms. Before the results of these experiments are reported, the destruction of native lignin in a single type of plant, harvested at different stages of growth, is illustrated from a typical experiment.

DECOMPOSITION OF LIGNIN IN OAT PLANTS OF DIFFERENT AGES

Oat plants were harvested from a uniform plot of ground, at three different stages of their development; namely, 59 days old, 86 days old, and 112 days

old or fully mature. At the last stage, only the straw and chaff were used. The results of the proximate chemical analysis of the plants at these three different stages are given in table 1. With an increase in age of plant, there was an increase in hemicellulose, cellulose, and especially lignin content, and a decrease in proteins, mineral substances, and ether-soluble and water-soluble materials.

The three oat plant preparations were air dried and cut into small fractions. Definite amounts, namely, 50-gm. portions of air-dry material containing 6 per cent moisture, were placed in 1-liter Erlenmeyer flasks. The young plant material, containing a higher nitrogen concentration than that required for its normal decomposition (20), received no additional nitrogen. The more mature plant preparations (86 and 112 days old) were treated with 2-gm.

TABLE 1

Chemical composition of oat plants harvested at different stages of growth

Per cent of dry material

	AGE OF PLANT		
	59 days	86 days	112 days
Total nitrogen	2.32	0 96	0.42
Ether-soluble fraction	4 42	2 98	2.26
Cold-water-soluble organic matter	14 85	7 88	3 45
Hot-water-soluble organic matter	4 84	3 23	2 48
Alcohol-soluble fraction	3 07	2 17	1 70
Hemicelluloses	15 25	17 35	19.27
Cellulose	24 55	34.50	39.10
Lignin*	6 70	11 70	15.70
Protein, water insoluble	7 55	2 34	1.70
Ash	9 62	9 05	4.20
Total accounted for	90.85	91 20	89 86

* Acid lignin, ash- and nitrogen-free

portions of $(NH_4)_2HPO_4$ or with 3-gm. portions of casein; some were left as controls, without nitrogen. All flasks received 2-gm. portions of $CaCO_3$ and 1-gm. portions of K_2HPO_4. The moisture content of the young plant material (59 days) was adjusted to 60 per cent, and that of the older material, to 75 per cent. The flasks were incubated at 28°C. for 47 and 125 days and analyzed (table 2). The results calculated on the basis of 100 gm. of the original dry material are presented in table 3.

The young oat plants decomposed so rapidly that after 47 days half of the total dry material has disappeared; when calculated on an ash-free basis, the percentage loss was even greater. The older the plant material, the slower was the rate of its decomposition. In the same period of time, in the absence of added nitrogen, only about a third of the 86-day-old plants was decomposed and less than a fifth of the mature plant material. The decomposition of the

TABLE 2

Decomposition of oat plants, harvested at different stages of growth

Per cent of residual dry material

Age of Plant ... days	59		86						112					
Treatment of Material	No nitrogen		No nitrogen		$(NH_4)_2HPO_4$		Casein		No nitrogen		$(NH_4)_2HPO_4$		Casein	
Period of Decomposition ... days	47	125	47	125	47	125	47	125	47	125	47	125	47	125
Total nitrogen	3 29	3.76	1.60	1 82	3.24	3.92	2.80	2.98	0.70	0.86	2.10	3.63	2.09	3.65
Ether-soluble fraction	3.69	3.30	1.82	1.08	2 12	1.59	2.20	0 55	2.03	1.18	1.77	0.87	1.57	0.53
Cold-water-soluble fraction	15.29	21.92	6.98	8.33	10 31	12.73	9.40	11 34	4.67	5.28	6.92	7.63	6.51	8.97
Hot-water-soluble fraction	5 44	6.80	4.27	6.07	6 80	8.90	6.77	9 66	2.61	4.53	3.80	9.11	4.82	12.86
Hemicelluloses	5 01	1.95	10.70	7.55	7.84	3.66	8.25	4.70	14.03	12.19	9.09	5.18	6.66	5.53
Cellulose	18.87	8 74	21.25	16 23	14.75	7.14	16.54	10 02	29.76	26.09	15.11	7.86	20.08	6.88
Lignin	9.47	7.09	13.35	13 21	14.47	14 89	12 45	12.63	15.91	16.56	20.89	21.08	20.10	16.86
Protein	11 87	15.00	7.02	8.50	13.43	17.88	12.96	13.62	3.23	4.24	8.67	18.76	9.38	18.76
Ash	26.17	33 80	22.15	26.90	27.25	32.50	26 70	31 80	12 30	14 10	21 95	27.30	18.50	24.45
Total accounted for	95 81	98 60	87.54	87 87	96 97	99 29	95 27	94 32	85 54	84.17	88 20	97.79	87 62	94.84

older plants was hastened considerably by the addition of available nitrogen; after 125 days the fully mature oat straw had decomposed to about the same extent as the very young plants when calculated on an ash-free basis. The favorable effect of available nitrogen upon the decomposition of the plant material is largely due to hastening the rapid destruction of the carbohydrate constituents, namely, the cellulose and hemicelluloses. In the case of the lignin, the addition of nitrogen did not have so controlling an influence upon its decomposition during the early stages. After 4 months, however, the amount of lignin decomposed was greatly influenced by the presence of available nitrogen. It is doubtful whether this was due to the direct influence of the nitrogen, since after 47 days very little of the added nitrogen was left in an inorganic form. Possibly, it may have been a result of a secondary influence of the synthesized nitrogenous compounds, which favored the development of lignin-decomposing organisms; it will be shown later that lignin-decomposing microörganisms prefer protein to inorganic salts as sources of nitrogen.

TABLE 3

Total amount of decomposition of oat plants, harvested at different stages of growth

On the basis of 100 gm. of original dry material

AGE OF PLANT	PERIOD OF DECOMPOSITION	SOURCE OF NITROGEN	TOTAL MATERIAL	TOTAL NITROGEN*	HEMICELLULOSES	CELLULOSE	LIGNIN
days	*days*		*gm.*	*mgm*	*gm.*	*gm.*	*gm.*
59	47	None	48.3	−619	12 66	14 80	1.80
59	125	None	56 3	−692	14 41	20 77	3 63
86	47	None	36.7	+53	10 58	21 05	3 25
86	47	$(NH_4)_2HPO_4$	39 3	+206	12 59	25.56	2 92
86	47	Casein	40 0	−120	12.40	24.58	4.23
86	125	None	37 4	+179	12 62	24 34	3 43
86	125	$(NH_4)_2HPO_4$	62 8	−302	15.99	31.84	6.16
86	125	Casein	52 4	−381	15 11	29.73	5.69
112	47	None	18 5	+151	7 84	14 85	2.73
112	47	$(NH_4)_2HPO_4$	39 7	+46	13.79	29.99	3 10
112	47	Casein	37 6	+44	15 11	26.57	3 16
112	125	None	27 1	+207	10.38	20.08	3.53
112	125	$(NH_4)_2HPO_4$	60 2	+225	17.31	35.97	7.31
112	125	Casein	62 1	+123	17 17	36 49	9 31

* + = gain, − = loss.

The comparatively rapid decomposition of the lignin in this experiment is due to the aerobic nature of the compost. Further, the lignin in oat straw seems to be readily subject to decomposition, since a much smaller amount of humus was left, especially in the presence of available nitrogen, as compared with composts of wheat and rye straw or of deciduous leaves (20).

PREPARATION OF PHENOL-LIGNIN

Phenol-lignin was prepared from the three samples of oat straw by treating the material with 3 parts of phenol containing 0.1 per cent of HCl. The lignin was dissolved in ethyl alcohol and precipitated by diluting and washing with water. The elementary chemical composition of the three samples of phenol-lignin from oat plants, as well as those from lowmoor and highmoor peat is reported in table 4. The carbon content varied within narrow limits, with an average, for the five preparations, of 69.47 per cent, very similar to that of phenol-lignin from spruce wood, which was 69.7 per cent. The nitrogen in the lignin preparations decreased with age of plant. The methoxyl content, however, increased with age of plant; this was true both of the plant itself and of the lignin prepared from it; in the case of the lignin, there was a slight decrease in methoxyl in the fully mature plant. The younger plant did not give so good a recovery of the methoxyl, pointing to a higher concentration of loosely bound methoxyl groups.

TABLE 4

Chemical composition of phenol-lignin from different plant materials and from peats

Per cent of dry material

NATURE OF MATERIAL	YIELD OF LIGNIN	CARBON CONTENT	NITROGEN	METHOXYL CONTENT	
				Original material	Lignin preparation
Oat plant, 59 days	11.6	70 75	0.82	1 71	4.71
Oat plant, 86 days	14 4	68 55	0 38	2.85	9.96
Oat plant, 112 days	27 3	69 69	0 21	3 41	7 95
Lowmoor peat	7 8	68.91	0 73	2.09	5.86
Highmoor peat	18.9	69 47	0 26	0 96	1 96
Oat plant, 112 days*	11.3	64.00	0.63	3 41	13.80

* Alkali lignin.

For the sake of comparison, the analysis of alkali-lignin prepared from the mature oat plant is also reported. Although the yield of this form of lignin is less than that of phenol-lignin, its methoxyl content is considerably higher.

DECOMPOSITION OF PHENOL-LIGNIN BY MICROÖRGANISMS

For the isolation of microörganisms capable of decomposing lignin, silica gel plates were prepared; casein was used as a source of nitrogen, and a dilute solution of certain minerals (K_2HPO_4, $MgSO_4$, $FeSO_4$), as well as $CaCO_3$, was also added. Phenol-lignin was dissolved in a small amount of alcohol, and the solution was added directly to the plates. The excess of alcohol was removed by drying the plates at 60°C. These plates were inoculated with soil and with enrichment liquid cultures containing phenol-lignin. Active development of a number of different fungi and bacteria took place on the plates.

The microörganisms were at first isolated in crude culture and were later reisolated on agar media containing phenol-lignin. They were tested for their capacity to decompose lignin, and only the active forms were utilized.

In the first experiment, phenol-lignin from spruce wood was used. Two-gram portions of the air-dry preparation were dissolved in 50 cc. ethyl alcohol, and the solution was added to 950 cc. water, with constant stirring. One gram of casein, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4 \cdot 7H_2O$, and 0.02 gm. $FeCl_3$ were also added. The medium was distributed into flasks, which were plugged with cotton and sterilized in flowing steam for 1 hour on two consecutive days.

TABLE 5

Decomposition of phenol-lignin by various fungi and bacteria in presence of alcohol

ORGANISM	27 DAYS OF INCUBATION		38 DAYS OF INCUBATION	
	Lignin left	Lignin decomposed	Lignin left	Lignin decomposed
	mgm.	*mgm.*	*mgm.*	*mgm.*
Control	153	...	148	...
Bacterium No. 5	20	133	26	127
Bacterium No. 8	15	138	30	118
Fungus No. 1	..	...	118	30
Fungus No. 2	12	141	10	138
Fungus No. 4	..	...	30	118
Fungus No. 7	25	130	12	136
Fungus No. 8	108	45	131	17

TABLE 6

Decomposition of phenol-lignin in alcohol-free medium

ORGANISM	CO_2-LIBERATED	NH_3-LIBERATED	LIGNIN LEFT	LIGNIN DECOMPOSED
	mgm. C	*mgm. N*	*mgm.*	*mgm.*
Control	16 7	...	178	..
Crude bacterium No. 5	42.9	4.7	122	56
Fungus No. 2	33.4	2.6	140	38
Fungus No. 7	41.9	3 8	111	67

Some of the flasks were left as controls, and others were inoculated with several of the cultures found capable of decomposing the lignin. The flasks were incubated at 28°C. for 27 and 38 days and were then analyzed for residual lignin. The method of determining the lignin consisted in extracting the culture with ethyl acetate and removing the extract through a separatory funnel; the extraction was repeated several times, until all the lignin was removed; the extracts were combined, filtered, placed in small weighed bottles, evaporated on a water bath, and dried to constant weight.

The results obtained (table 5) show that all the organisms tested were cap-

able of decomposing the phenol-lignin actively but to a varying quantitative extent. The three most active forms were found to be fungi Nos. 2 and 7 and bacteria Nos. 5 and 8. Different cultures of the same organisms behaved similarly.

A study was now made of the decomposition of phenol-lignin in the absence of alcohol. The alcoholic solution was prepared as before, and the alcohol was removed by evaporation under reduced pressure. The nutrient substances were added, and the solutions were made up to volume and distributed into flasks. These were sterilized, inoculated, and incubated for 46 days. The CO_2 liberated and the ammonia produced (from the casein) were also determined (table 6). The three different organisms tested were found to decompose phenol-lignin also in the absence of the solvent; however, the amounts decomposed were considerably less.

In order to test the effect of another solvent, 5 gm. of lignin was dissolved in 50 cc. acetone, and the solution was suspended in water; the acetone was removed by evaporation, at reduced pressure, and the solution was made

TABLE 7

Decomposition of phenol-lignin in acetone-free medium

ORGANISM	LIGNIN LEFT	LIGNIN DECOMPOSED
	mgm.	*mgm.*
Control	277	..
Bacterium No. 5 (crude culture)	190	87
Bacterium No. 5 (pure culture)	230	47
Fungus No. 2	238	39
Fungus No. 7	214	63

up to 1500 cc. with water. The nutrients were added, and the medium was distributed into flasks, which were sterilized, inoculated, and incubated for 25 days (table 7). In this experiment, as well, the various organisms were found capable of decomposing phenol-lignin.

Two cultures were now selected for testing the decomposition of the various phenol-lignin preparations, namely, a crude culture of a Fusarium and one of an Alternaria; ammonium phosphate and casein were used as sources of nitrogen. Acetone was employed as the solvent for the lignin and removed by evaporation under reduced pressure. The cultures were incubated for 40 days and the residual lignin determined (table 8). Some lignin was decomposed in all cases. This experiment was repeated using alcohol as a solvent for the lignin, since traces of acetone left in the medium were found to have a somewhat retarding effect upon the growth of the organisms. Much larger amounts of lignin were decomposed, both in the presence and in the absence of the solvent. It is of interest to note that in this experiment there was no increase in the resistance of the lignin to decomposition with an increase in maturity of the oat plant from which the lignin was isolated. This is

comparable to the behavior of the lignin in the native state, namely, in the oat plants, when the latter undergo decomposition.

In the final experiment, larger quantities of lignin were subjected to decomposition by two crude cultures of fungi. Six 8-gm. portions of phenol-lignin from the mature oat straw were dissolved in 200-cc. portions of alcohol and dispersed in 2 liters of water. The alcohol was removed by evaporation

TABLE 8

Decomposition of various lignin preparations by two crude cultures of soil microörganisms in acetone-free medium

NITROGEN SOURCE	ORGANISM	LIGNIN PREPARATION, MGM.									
		Oat plants, 59 days old		Oat plants, 86 days old		Oat plants, 112 days old		Lowmoor peat		Highmoor peat	
		Left	Decomposed	Left	Decomposed	Left	Decomposed	Left	Decomposed	Left	Decomposed
$(NH_4)_2HPO_4$	Control	141	..	159	.	189	.	133	..	133	..
	Fusarium	104	37	147	12	163	26	.	..	109	24
	Alternaria	120	21	147	12	167	22	112	21	108	25
Casein	Control	128	.	158		176	.	136	.	129	.
	Fusarium	108	20	107	51	164	12	63	73	93	36
	Alternaria	110	18	127	31	144	32	92	44	100	29

TABLE 9

Decomposition of phenol-lignin from oat straw by soil microörganisms

PERIOD OF DECOMPOSITION	CONTROL		FUSARIUM SP.			ALTERNARIA SP.		
	"Growth", weight	Residual lignin	"Growth", weight	Residual lignin		"Growth", weight	Residual lignin	
				Left	Decomposed†		Left	Decomposed
days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
30	0.926*	2.790	2 094	2 525	0 173	...	.....	.....
98	.....	2.606	. ...	.....	...	1.791	2.201	0.497
230	.....	.....	1 151	2.171	0 527	1 882	2 019	0 679

* Consists almost entirely of casein, as shown by the fact that it contained more than 10 per cent nitrogen.

† Difference between average lignin content of culture and of the two controls.

of 400 cc. of the liquid under reduced pressure. All portions were combined and made up with water to 9600 cc.; 800-cc. portions of this lignin suspension were placed into 3-liter Fernbach flasks; each flask received 1 gm. of casein, 1 gm. K_2HPO_4, 0.5 gm. $MgSO_4$, and 0.01 gm. $FeCl_3$. Five flasks were inoculated with the crude culture of the Fusarium and five with the Alternaria (fungus No. 7); two flasks were left as controls. At the end of varying periods

of incubation at 28°C., the cultures were removed and acidified with HCl. The precipitate was either filtered off through paper or centrifuged, washed, and extracted with ethyl acetate. The insoluble residue was designated as "growth"; the soluble portion was evaporated, dried and weighed, and recorded as "residual lignin" (table 9).

Definite decomposition of the lignin took place, as shown by its gradual disappearance in the cultures. This was accompanied by the transformation of some of the casein nitrogen into microbial protein. The concentration of phenol was determined in the residual lignin and was found to be 15.88 per cent in the control and 15.67 per cent in the material upon which the fungus had grown, leading to the conclusion that the phenol was not split from the molecule and that the lignin complex was attacked as a whole. Although in the older cultures there was an increase in the soluble organic matter left in solution after the removal of the lignin, no definte lignin derivatives could be demonstrated. No acid formation could be detected.

DISCUSSION

Although in the decomposition of plant residues in composts and in soils the lignin constituents are the most resistant and tend to accumulate, they are not absolutely resistant and are attacked to a greater or lesser extent by a number of organisms. The nature of the plant material is of importance in this connection; the lignins in the straw of wheat and rye and in the leaves and needles of trees are much more resistant to decomposition than the lignins in alfalfa or in oat plants. This phenomenon, as well as the greater resistance of the lignin in mature than in young plants, has an important bearing upon the problem of formation and accumulation of humus. The results presented in this paper confirm similar observations already reported from this laboratory. However, a new fact was brought out here, namely, the significance of organic sources of nitrogen in the decomposition of the lignins in plant materials. This phenomenon also has a bearing upon such problems as mushroom production, where the organism in question feeds primarily upon the lignin derivatives in the compost.

An attempt was further made to decompose lignin isolated in a free state from the other plant constituents. In this respect, phenol-lignin proved to be very convenient, since it could easily be dispersed, by means of various solvents, in a liquid medium. Only in that state could it be subjected to decomposition. A number of common soil fungi were found capable of bringing about this process. In view of the fact that the important point was to demonstrate the capacity of soil organisms to attack isolated lignin, the specific nature of these organisms has not as yet been studied.

An attempt to determine the mechanism of the breakdown of the lignin complex failed. One can hope to do this only after the chemistry of lignin is better understood. The only facts that can be emphasized at present are the following: 1. Even those organisms that are capable of attacking isolated

lignin do it very slowly; 2. the lignin is utilized very efficiently, accompanied by the liberation of only very little CO_2, while the major part of the carbon is transformed into microbial cell substance; 3. the microbes utilizing lignin seem to build up cell substance which has an appreciable concentration of this complex. The second fact finds substantiation in the growth of mushrooms upon lignin-rich composts, whereas the third finds confirmation in the high lignin content of wood-destroying fungi (16). The problem has been further complicated by the fact that when alcohol was used as a solvent for the lignin, it could not be all removed from the dispersed medium; hence there is a possibility that some of the growth of the organisms may have been due to the utilization of the traces of alcohol left in the medium.

SUMMARY

A study has been made of the decomposition of lignin in a native state, namely, in oat plants harvested at different stages of growth, and of lignin isolated in a free state, in the form of phenol-lignin.

In accordance with results of other investigators and with those obtained in this laboratory, it was found that lignin in plant materials was more resistant to decomposition than the other groups of plant constitutents. However, it underwent a gradual, even if slow, decomposition.

Several organisms were isolated from the soil and found capable of decomposing phenol-lignin from spruce wood, oat straw, and peat. In order to bring about the decomposition of lignin by these organisms, it had to be dispersed in water. This was accomplished by dissolving the lignin in alcohol and adding it to an excess of water. The solvent was then removed by evaporation at reduced pressure.

Except in a few preliminary experiments, where crude enriched cultures of bacteria and fungi were employed, the rate of decomposition of the lignin was slow.

No product of lignin decomposition could be isolated or demonstrated.

The efficiency of the carbon utilization by the microörganisms attacking the lignin was very high.

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A COMPARISON OF THE SPECIFIC GRAVITY BALANCE AND THE PIPETTE METHODS OF DETERMINING DENSITY OF SOIL SUSPENSIONS

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In an experiment to determine the effect of cultural treatment of field soils on their permeability to irrigation water, it was thought desirable to make a study of the size distribution of individual soil particles and aggregates which remain more or less stable in a water suspension. This paper reports some tests on the use of a specific gravity balance as compared with the pipette for studying the size distribution as measured by settling velocities. A report of a previous investigation on dispersed soil in which the theory of the method has been discussed has been made by others.[1]

The balance used was lent to us for this purpose by Christian Becker, Inc. It (pl. 1) is an adaptation of their standard chainomatic balance especially designed for this purpose by mounting it on a stand with a rack and pinion to raise and lower the balance and plummet.

The procedure used in making a reading was to lower the plummet slowly into the liquid until its center of gravity was 15 cm. below the surface of the water. A rate of about 1 cm. per second was found to be a convenient speed. When lowered to the proper depth, the pointer was watched as the beam was lowered on the knife edge and was allowed to swing only far enough to indicate the direction of swing. The beam was then raised, the weights were readjusted, and the process was repeated until, when balanced, the pointer remained at zero. This static method was found to be necessary to prevent the plummet from moving up and down in the liquid and stirring it, which would interfere with the free falling of the particles in suspension. After each reading the plummet was raised out of the liquid at the same speed as that at which it was lowered. It was then rinsed with distilled water to clean it for the next reading. With a little practice it soon became easy to make readings in this manner.

A series of tests was made on dispersed soil. The results are shown in table 1. Columns 2, 3, and 4 show the percentages of soil suspension at the settling times shown in column 1. Ellipsoidal plummets, 10 cm. long, having the

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volumes indicated, were used. The means of the measurements obtained with plummets of this shape are shown in column 5. In column 6 are the results obtained with a spherical plummet. In column 7 are the results ob-

TABLE 1

Suspension densities at the end of various settling times, as measured by the specific gravity balance with different sized plummets and by the pipette method, 15 cm. below the surface

Densities expressed as per cent of the initial suspension density

TIME	ELLIPSOIDAL PLUMMETS OF DIFFERENT VOLUMES				SPHERICAL PLUMMET 10.15 cc.	PIPETTE
	46.35 cc.	14.14 cc.	4.53 cc.	Mean for ellipsoidal plummets		
min.						
1	56.38	58 18	56.60	57.72	34 95	56 85
3	50 60	52.00	49.30	50.63	47 20	50.20
5	44.40	46.80	44.70	45.30	44.83	45 85
10	37.32	39 70	37.20	38.07	40.10	38 70
20	33.15	33.96	32.60	33 23	33 60	33 10
30	29 82	31.50	29.54	30.28	29.65	31 15
hrs.						
1	26.25	25.49	24.04	25.26	24.83	26 15
2	22.03	22.32	19 42	21 26	22.77	22.50
4	18.40	18.84	18.16	18.47	19.68	19 55

TABLE 2

Replicate determinations on three soils of suspension densities 15 cm. below the surface, at various settling times, as measured by the specific gravity balance

Densities expressed as per cent of the initial suspension density

TIME	YOLO LOAM (SLACKED)				YOLO LOAM (DISPERSED)				SACRAMENTO ADOBE CLAY (SLACKED)				
	1	2	3	Aver-age	1	2	3	Aver-age	1	2	3	4	Aver-age
min.													
1	34 1	32.5	35.4	34.0	58.4	60.2	61.0	59 9	15.86	15.05	15.00	15.08	15.25
3	19.4	20 6	21.3	20.4	51.7	52 0	52.3	52 0	11.32	11.74	11.28	11.26	11.40
5	16.5	15 6	15 7	15.9	45.0	46 4	49 0	46 8	9.81	9 22	9.28	9.92	9.56
10	10.9	11.0	10.6	10.8	37 1	39 6	42.5	39.7	7 87	7.82	7 53	7.81	7.76
20	7 6	7 6	8 8	8 0	31.5	33 5	36.9	34.0	6.80	6.25	6 21	6.05	6.33
30	5.8	6.2	6 4	6.1	28 8	32.4	33.4	31.5	6.01	5 80	5.82	5.42	5 76
hrs.													
1	4.1	4.5	4.2	4.3	24.3	26.8	28.6	26 6	4.80	4.44	4 44	4 24	4 49
2	1.9	1.9	1.8	1.9	20.5	22.3	24.1	22 3	3 97	3.61	3.83	3 74	3 79
4					17 2	18.3	21.0	18 8					

tained with the pipette method. All readings were made at about 25°C. The center of each plummet was lowered to the same depth as that at which the pipette samples were taken. The difference between the buoyancies of the

suspension and of the pure water, together with the volume of the plummet, furnished sufficient information for a calculation of the average amount of material in suspension per unit of volume at the same depth as the center of the plummet. It was assumed that this represented the density of suspension at the depth where the center of the plummet was located. It is obvious that the settling velocity can be calculated from these data if desired.

TABLE 3

Suspension densities 15 cm. below the surface at various settling times as measured by the specific gravity balance and by the pipette

Densities expressed as per cent of initial suspension density

TIME	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette
min.										
1	74 9	82.3	71 7	76.7	75.0	81 1	77 6	81.9	74 8	76.7
3	70 8	70 6	66.3	63.3	72 0	68 0	69.7	64 7	70.7	67.2
5	63 6	64.2	59 8	59 6	64.8	62 6	62.4	59.9	62 9	59.3
10	53 5	55 0	52 8	51 6	56.5	54 5	55 4	52.6	55.0	53 5
20	46 8	46 3	45 1	47.6	47.5	45.4	47 0	45 8	47 4	46 5
30	41 8	44 1	40 7	41.9	42.7	42.3	43.3	41.1	43.0	42 0
hrs.										
1	35 6	36 3	34.5	36 0	35 8	34.8	36.7	35 8	36 7	36.0
8	20 3	22 3	21 3	23 1	21.7	21.0	22 9	23 0	22 4	23 3
24	14 3	19 0	15 2	19.1	15.7	17.4	17 9	18 0	16 7	18.0
48	11 0	14 5	12 2	16.0	12.4	15.2	13.8	15.0	13.5	15.5

TIME	SAMPLE 6		SAMPLE 7		SAMPLE 8		SAMPLE 9	
	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette	Plummet	Pipette
min.								
1	75 6	77 8	57 4	61 1	75 3	80 4	44 6	48 1
3	70 4	68 1	49.2	59 4	75 1	77 7	42 5	39.7
5	69 4	61 6	45 2	45 6	73.4	76 4	38.0	34.2
10	56 2	55 1	38.9	40.3	70 0	73.4	32.6	29 8
20	47 6	47 3	34.7	35 0	66 7	70.2	27 4	25.0
30	43 3	43 8	32 2	33.2	64 4	67 5	25.3	24 2
hrs.								
1	38 7	37 7	27 6	28 2	60.9	64 6	20.8	21.3
8	22.0	24 8	17.5	19.1	54.9	55.7	13.2	14.2
24	15 6	19 6	13 3	15.2	49 5	50.3	10.7	9.35
48	11.9	15.8	10.8	12.0	47.4	48.5	9.6	9.30

The size of the ellipsoidal plummet within the range used here seems to make little difference in the readings obtained, and the data agree closely with those obtained by the pipette method. When the spherical plummet was used, material settled on the top of it while the first two readings were being taken. This caused these readings to be in error by a large amount. Later

readings, however, agree very well with the pipette results as well as with those obtained by the ellipsoidal plummets.

Another series of tests was run to determine how closely replicate determinations agreed. These results are presented in table 2. The tests were made on Yolo loam slacked in water, on Yolo loam dispersed with an electrical stirrer for 10 minutes without the aid of chemical dispersing agents, and on Sacramento adobe clay slacked in water. The reasonably good agreement among replicates is evident.

Since the pipette is usually regarded as the most accurate method of determining density of suspension, a further comparison of the balance and the pipette determinations on a number of soils was made. The results are given in table 3. The agreement here again is close.

TABLE 4

Suspension density 15 cm. below the surface at various settling times as measured by the specific gravity balance using suspensions having different initial suspension densities

Densities expressed as per cent of the initial suspension density

TIME	AIKEN CLAY LOAM			YOLO LOAM			SAN JOAQUIN SANDY LOAM		
	Per cent of concentrations			Per cent of concentrations			Per cent of concentrations		
	1	2	4	1	2	5	1	2	5
min.									
1	75 60	74 97	78 60	50 85	57 38	57 57	39 75	44 21	42 60
3	75 27	74.77	75 94	50 20	49 24	49 50	39 66	40 12	37 65
5	74 25	72 53	75.00	44.92	45.23	43.60	36 18	36 65	35 03
10	70 10	69.34	71 76	39 00	38.85	38 95	30 43	30 66	29 76
20	65.90	65.58	68 70	32 70	34 72	34 16	25 96	25 87	24 60
30	63 04	64.00	65.42	29 47	32 20	31 33	22 50	23 32	22 35
hr.									
1	59.42	60 20	61.72	25 22	27 60	27.18	20 18	19 72	18 90

It was recognized that the concentrations of the suspension might affect the determinations. The results of tests to determine whether this effect is appreciable are shown in table 4. Determinations at different concentrations on a given soil agree closely. Apparently concentrations as high as 5 per cent are not great enough to interfere with the free fall of particles.

The range of settling velocity which can be measured by this method is limited by the time required to make the first reading. One minute is about as soon as a reading can be made. This is also the case with the pipette method. It is difficult, therefore, to measure settling velocities greater than 0.25 cm. per second. A large part of the slacked soil had settled below the center of the plummet at the end of 1 minute, as shown in table 2. It is clear, therefore, that the balance is not suited for studying settling velocities greater than 0.25 cm. per second. The specific gravity balance is suitable for use over

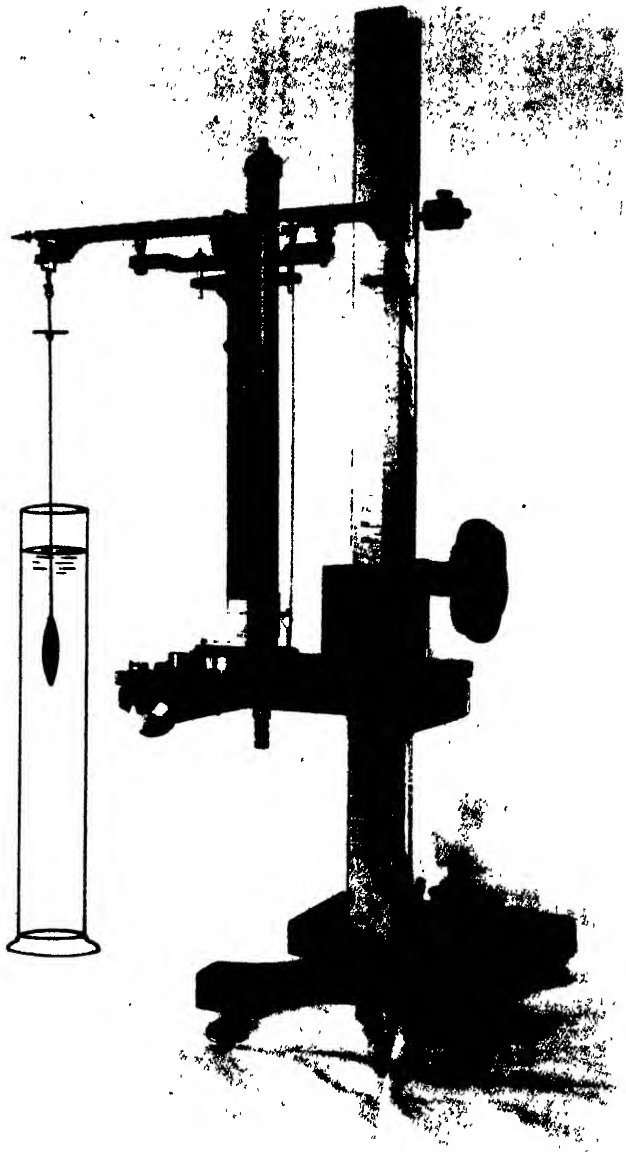
about the same range of settling velocities as the pipette method and requires considerably less working time to make the measurements.

SUMMARY

A comparison was made of the specific gravity balance and the pipette methods for determining the density of soil suspensions. Results obtained with different sized ellipsoidal plummets closely agree with one another and with the data obtained by the pipette method but do not agree with data obtained with a spherical plummet. Replicate determinations made with the specific gravity balance on a given soil agree closely. Initial suspension densities as high as 5 per cent were not great enough to interfere with the free fall of particles. The specific gravity balance is suitable for use over about the same range of settling velocities as the pipette method but requires considerably less time to make a measurement.

PLATE 1

SPECIFIC GRAVITY BALANCE USED IN MAKING SOIL SUSPENSION DENSITY DETERMINATIONS



SOIL SAMPLERS

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In studying some of the physical properties of soils, with particular reference to those influencing the movement and availability of water, it is often desirable to obtain soil samples the natural structure of which is undisturbed. Two types of cylinders have been devised and used with success by the writer in the soils of the Duke Forest.

Figure 1 shows a cylinder for obtaining samples of undisturbed soil for the determination of volume-weight, air space, and water-holding capacity. The unit consists of a cutting cylinder made of 5-inch high-pressure steel pipe machined down to the specifications indicated on the diagram and an inner cylinder made of 5-inch seamless brass tubing. The brass inner cylinder is held in place by a ring and pin while the unit is being driven into the soil with a sledge hammer. A counter-sunk steel plate or a block of wood placed on the cylinder prevents it from being battered by the hammer. The cutting edge should be case hardened. Constructed as indicated, the soil cylinder has a volume of 600 cc., a depth of slightly less than 2 inches, and an inside diameter of just under 5 inches.

Lids pressed from 10-ounce copper are used on the brass cylinders for transporting samples from the field to the laboratory. Squares of copper window screen, 16-mesh, have been used effectively over one end of the cylinders while the samples are being saturated with water, and later drained, for the determination of air space and water-holding capacity Filter paper cut from paper toweling can be used to keep the soil from passing through the screen. Because of the great variation of volume-weight and allied measures of undisturbed soil, due to the presence of rocks, roots, and animal burrows, it is usually necessary to take a large number of samples in the field. The writer uses 50 brass inner cylinders with two cutting cylinders.

Figure 2 gives the dimensions of a cutting cylinder devised for taking samples of undisturbed soil for making determinations of the permanent wilting percentage. Cardboard ice-cream containers of one-pint capacity are placed in an inverted position in the cutting cylinder before it is driven into the soil. The bottom of the container is cut off in the greenhouse, and seeds of the plant to be used for determination of the wilting percentage are planted. The surface is not sealed until the plants have become well established and the moisture content of the soil has been reduced to near the wilting percentage. At that

time it is good to moisten the surface soil with water to a depth of 1 cm. and then seal the top of the container with paraffin to a depth of about 5 mm. The

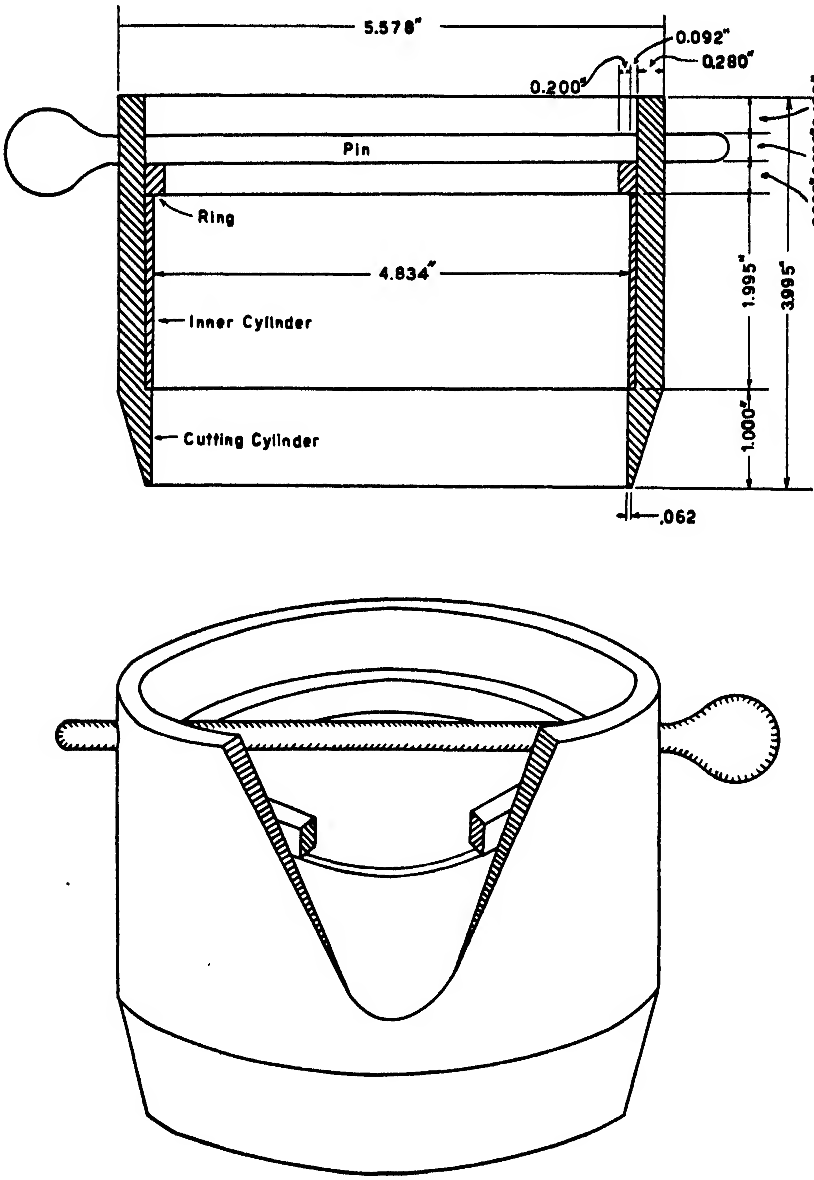

FIG. 1. SAMPLER FOR OBTAINING UNDISTURBED SOIL FOR DETERMINING VOLUME-WEIGHT, AIR SPACE, AND WATER-HOLDING CAPACITY

writer has found ordinary technical paraffin quite satisfactory when oats and sunflower are used as phytometers. The paraffin melts at about 54°C. If

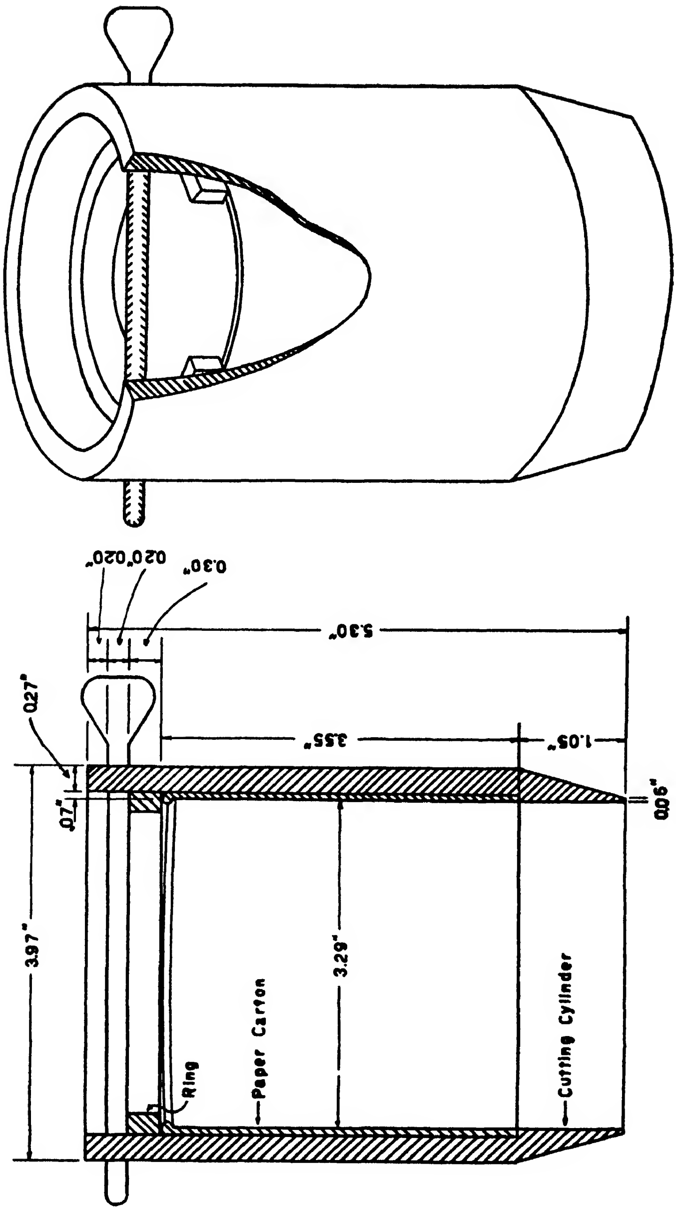

FIG. 2. SAMPLER FOR OBTAINING UNDISTURBED SOIL FOR DETERMINING THE PERMANENT WILTING PERCENTAGE

the temperature of the paraffin is not over 60°C. when it is applied it will solidify as soon as it contacts the moist soil and will not injure the plants. The containers can be boiled in paraffin before they are used and then coated on the outside after the field samples are obtained.

Either of the two cylinders described can be used for obtaining samples of undisturbed soil for moisture equivalent determinations. Sub-samples are cut out to fit the moisture equivalent cups in the laboratory.

TRANSFORMATION OF NITRATE IN WATER-LOGGED SOILS

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In comparison with the voluminous literature on the changes of nitrate in dry cultivated soils, that dealing with similar transformations in water-logged soils is limited. Among the early workers, Warington (16) was the first to notice that nitrate added to the soil could not be completely recovered when the soil remained covered with water. Nagaoka (9) observed that nitrites were formed as a result of heavy dressings of nitrate to rice fields. Daikuhara and Imasaki (2) have shown that denitrification takes place very rapidly in swamp soils, leading to the loss of nitrogen and to the formation of toxic nitrites. Kelley (8) has observed considerable fall in nitrate content in water-logged soils. Harrison and Aiyer (6), from an analysis of the gas evolved from swamp paddy soils, have concluded that the nitrate formed during the dry season is quickly denitrified and is of no use so far as the cultivator is concerned. More recently, Subrahmanyan (12) observed that water-logging resulted only in slight diminution in nitric nitrogen and that there was no formation of nitrite and no denitrification. Janssen and Metzger (7), on the other hand, found that in flooded soils nitrates are rapidly lost. Similar results were obtained by Prescott and Piper (10) who, working with Australian soils, observed rapid disappearance of nitrate at moisture contents above 18 per cent.

The foregoing review shows that although the previous workers were practically unanimous in their observation that water-logging of soil leads to considerable loss of nitrate, no attempt has been made to account for this loss, except the observations made by a few workers that denitrification takes place under conditions of water-logging.

The study of the changes of nitrate in water-logged soils is important from both the scientific and the practical points of view. No soil constituent shows such great fluctuations as nitrate and none is so susceptible to external influences. Under the widely differing chemical and biological conditions existing in water-logged soils, the course of the transformation of nitrate will probably be different from that in dry cultivated soils. From a practical point of view, the importance is even greater. Water-logging of soils, though comparatively less frequent in cold countries, is a common phenomenon in the tropics where millions of acres of dry land with the accumulated nitrate pass into the water-

[1] Thanks of the senior author are due to the Imperial Council of Agricultural Research for maintaining his salary.

logged state during rains. For the cultivation of rice, vast areas have to be maintained under swamp conditions for months. It is desirable, therefore, to obtain further knowledge of the effect of water-logging on the change of nitrate in soils; with this object in view the present investigation was undertaken.

METHODS OF ANALYSIS

Total nitrogen. In the absence of nitrate, total nitrogen was determined by the routine Kjeldahl method. A modification of Davisson-Parson's method (3) was used in estimating total nitrogen in water-logged soils containing nitrate. The soil-water mixture was distilled with 2 gm. of MgO and 2 gm. of Devarda's alloy, and the distillate was collected in 0.02 N H_2SO_4. The ammonia absorbed by acid was determined by back titration, using methyl red as indicator. The residue after distillation was digested with H_2SO_4 in the usual way. The sum of nitrogen in the first distillate and that in the residue, corrected for the nitrogen in the reagents used, gave the total nitrogen content of the soil.

Ammoniacal nitrogen was determined by Bengtsson's method (1). Nitric nitrogen was estimated by Devarda's alloy method.

Organic carbon was determined by the wet combustion method developed by Friedemann and Kendall (5). Correction was made for carbonate carbon where necessary.

Carbonate carbon was estimated by Collin's calcimeter.

Carbon dioxide evolved from soil was determined by absorption in 0.1 N $Ba(OH)_2$ solution and back titration against 0.05 N oxalic acid using phenolphthalein as indicator.

Soil organic matter. Analysis of soil organic matter was carried out according to the method developed by Waksman and Stevens (14).

Number of bacteria and actinomyces was determined by plating on Thornton's agar (13).

pH was determined by means of the quinhydrone electrode using soil-water mixture in the ratio 1:2.5.

SOIL SAMPLES USED

To observe the general effect of water-logging on nitrate, a few preliminary experiments using different soils were carried out. A known amount of nitrate was added to each soil, which was then water-logged and incubated. Considerable loss took place in every case. Out of this list, the following two, obtained from unmanured paddy fields, were selected for detailed study: Dacca farm soil—lateritic in type and acid in reaction (pH 5.1); Faridpur soil—a sandy loam alluvial soil alkaline in reaction (pH 8.3).

Samples of soil (0–6 inches) were air dried, passed through a 1-mm. sieve, and then preserved in bottles for use in the experiment.

EFFECT OF WATER-LOGGING ON THE NITRATE AND AMMONIA CONTENTS OF SOIL

By the addition of 100 cc. of distilled water in 250-cc. Erlenmeyer flasks, 50-gm. portions of soil were water-logged. The flasks were then divided into two sets: the first remained untreated and served as a control, and the second set received a known amount of nitrate as KNO_3. A third set of flasks each containing 15 gm. of soil and 100 cc. of distilled water, with or without added

TABLE 1

Changes in ammonia, nitrate, and total nitrogen in water-logged soil exposed to light

Results expressed as p.p.m.

Faridpur soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N		SOIL + 4.144 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	2.2	10.6	2.2	52 1	2 2	93.5
3	6.6	2.8	6.4	32 2	6.2	72.9
7	3.6	0.3	3.1	14.6	1.1	49.7
15	1.1	0.4	0 0	0.3	0.0	18.5
30	0.0	0.1	0.0	0.0	0.0	0.0
	Total N		Total N		Total N	
Start	639		680		722	
30 days	727		675		722	

Dacca soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	11 2	16.0	11.2	57.5
3	13.9	5.3	14.4	42.0
7	15.3	1.0	17.0	24.4
15	8.0	1.3	7.8	18.5
30	2.3	0.9	0.0	19.4
	Total N		Total N	
Start	961		1002	
30 days	951		989	

nitrate, was also set up and used for the determination of total nitrogen. The flasks were exposed to sunlight in the pot culture house. After 5 to 6 days' exposure, the evolution of gases from the soil surface was noticed in every flask. A few days later it was further observed that a greenish matter was appearing in the flasks containing Faridpur soil. This green growth gradually increased in bulk, and in a month the entire surface of the liquid was covered with a thick

growth of it. On the other hand, there was very little growth in the flasks containing Dacca soil. At intervals of 3, 7, 15, and 30 days, duplicate flasks from each treatment were removed for the determination of ammonia and nitrate. As nitrite was present only in traces, it was not estimated separately. Total nitrogen was determined only at the end of the experiment. The results are given in table 1.

TABLE 2

Changes of ammonia, nitrate, and total nitrogen in water-logged soil kept in the dark

Results expressed as p.p.m.

Faridpur soil

TIME IN DAYS	CONTROL		SOIL + 2.072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	2 2	10.6	2 2	52.1
3	7 0	2 1	7 3	27.7
7	4 2	2 6	3 4	9 2
15	4.2	1 5	1 8	1 8
	Total N		Total N	
Start	639		680	
15 days	635		668	

Dacca soil

TIME IN DAYS	CONTROL		SOIL + 2 072 MGM. NO_3—N	
	NH_4—N	NO_3—N	NH_4—N	NO_3—N
0	11.2	16.0	11.2	57.5
3	13 4	7.0	13.7	43 7
7	16.4	2.2	18 0	24.1
15	16 4	2 0	14.1	17.6
30	4 3	1.3	0 0	25 8
	Total N		Total N	
Start	961		1002	
30 days	946		986	

The appearance of the green algal growth in some of the flasks has introduced a complicating factor, since a portion of nitrate and ammonia might be used up for its body formation. To eliminate this factor, the experiment was repeated in the dark. The results (table 2) show that toward the latter part of the experiment a little ammonia and nitrate was assimilated by the growth.

The effect of water-logging, in general, is similar in both soils. Both have suffered heavy loss of nitrate and loss of ammonia after an initial rise but no great loss of total nitrogen. In spite of this resemblance, a difference will be

observed in their behavior when the figures for nitrate in the treated soils (soil + 2.072 mgm. NO_3–N) are compared. Whereas in Faridpur soil the added nitrate had nearly completely disappeared within the period of experiment, in Dacca soil, after a portion had been lost, no further reduction in the amount of nitrate took place; in fact, in the experiment in the dark, a rise from 17.6 p.p.m. on the fifteenth day to 25.8 p.p.m. on the thirtieth day was noticed. The behavior of Dacca soil is significant and shows that under certain conditions nitrate may survive in water-logged soils.

The loss of ammonia after an initial rise is in accord with the results of an investigation on the changes of ammonia in water-logged paddy soils carried out in this laboratory for three successive crop seasons. In all the 32 plots

TABLE 3

Effect of nitrate on the evolution of carbon dioxide from water-logged soil

Mgm. of carbon dioxide evolved from 300 gm. of soil

	0–2 DAYS	2–4 DAYS	4-7 DAYS	7–10 DAYS	10–13 DAYS	13–15 DAYS	15–18 DAYS
Untreated soil	10 2	18 1	54 9	47 1	30 5	29 2	21 2
Soil + nitrate (12 mgm. NO_3—N)	16.4	22 2	68 0	51 4	32 0	30 0	21 0

TABLE 4

Effect of nitrate on the numbers of bacteria and actinomyces in water-logged soil

Numbers of bacteria and actinomyces as millions, per gram of air-dry soil

DAYS AFTER WATER-LOGGING	BACTERIA AND ACTINOMYCES IN	
	Untreated soil	Soil + nitrate
3	3 75	6 0
7	5.75	12 0
10	3.45	6 0
18	2.55	2 5

selected for experiment, it was found that the ammonia content began to rise soon after water-logging, reached the maximum in about 6 weeks, thereafter remained stationary for about a month, and then fell. Similar observations were made in pots. In the foregoing experiments, the amount of nitrate lost was so much greater than the amount of ammonia formed that the reduction of nitrate to ammonia does not appear to be a reaction of importance in water-logged soils.

Except in control Faridpur soil (experiment in light), where there was a distinct increase, total nitrogen remained practically unchanged in all cases, the small differences observed being all within the limits of experimental error. As the results obtained at the end of the experiments, however, are consistently, though not significantly, lower than those at the start, it is believed that some

small loss of nitrogen occurred in the course of the experiment. But even then, the loss of nitrogen accounts for only a portion of the nitrate lost. The method for determining total nitrogen is not sufficiently accurate to detect losses of small amounts of nitrogen which may have occurred by dentrification. The increase in nitrogen in Faridpur soil is due to the fixation of atmospheric nitrogen by the green growth previously described (4).

From the foregoing observations, it would appear that the loss of nitrate in water-logged soils is not due to its reduction to ammonia, though a portion may be lost by denitrification. Evidently, the main loss is brought about by some other agency. It is presumed that nitrate is assimilated by microörganisms in the presence of the energy material supplied by the soil. If this view is correct, the loss of nitrate should be attended by a rise in bacterial numbers and an increased production of carbon dioxide. The results in tables 3 and 4 support this view. The experiment on which these results are based was performed with Dacca soil only, since the high content of carbonate in Faridpur soil made it unsuitable for use in an experiment on the production of carbon dioxide because of the absorption of the gas by the base, particularly during the early period of water-logging.

At first, carbon dioxide evolved in greater amounts from the treated soil, but the rate soon diminished until, after 13 days, nearly equal amounts were given off by the control and by the treated soils. Bacterial numbers also showed similar fluctuation. In the treated soil, the number was much higher in the beginning but after 10 days it dropped to the same range as that in the control. The rapid rise of bacteria for a few days in the beginning, followed by a decrease afterward, clearly explains why nitrate added to Dacca soil suffered loss only to 15 days and showed no further loss thereafter (tables 1 and 2). It is inferred that this slowing down of the bacterial activity is due to the exhaustion of the available energy supply of the soil.

ASSIMILATION OF NITRATE BY MICROÖRGANISMS IN RELATION TO SUPPLY OF ENERGY MATERIAL

As the supply of energy material is one of the main factors limiting the growth of the microörganisms, it is obvious that in a soil rich in energy material, nitrate will be assimilated to a greater extent than in one with a poor supply. The nearly complete disappearance of nitrate in Faridpur soil, compared with the limited loss in Dacca soil, indicates the relatively greater supply of energy in the former. If the excess of energy is reduced, it will probably behave in the same manner as the other, i.e., it will show only a limited loss of nitrate. This was tested in the following experiment.

A portion of Faridpur soil was incubated under optimum conditions of temperature and moisture until the maximum accumulation of nitrate took place. At this stage, the supply of nitrate was much in excess of the energy supply, otherwise the former could not have accumulated. The soil was then water-logged, and the changes of nitrate were observed. For comparison,

a portion of fresh soil (not incubated) was similarly water-logged after addition of nitrate and analyzed at stated intervals. The results are given in tables 5 and 6.

The influence of the energy materials on the transformation of nitrate in water-logged soil is clearly shown in table 6. In the fresh soil, containing much energy material, nitrate rapidly disappeared, until at the end of a month practically none was left. On the other hand, incubation resulted in the decomposition of much of the energy material; consequently the loss of nitrate in this case was small.

In view of the fact that under normal soil conditions the supply of energy material is a much more important limiting factor for the growth of microorganisms than is the nitrogen supply, it may be difficult to accept the hypothesis that nitrate added to water-logged soils is assimilated by the microörganisms. Table 5 shows that, in Faridpur soil, the quantity of nitrate had considerably increased within the first 15 days, indicating the presence of very

TABLE 5

Production of nitrate in Faridpur soil on incubation

Incubation period........ *days*	0	15	30	45	60	75*	90	105
NO_3—N .... *p.p.m.*	5.7	24.2	33.7	52.4	59.5	60.1	61.2	62.4

* After 75 days the incubated soil was water-logged.

TABLE 6

Effect of water-logging on the nitrate contents of fresh and incubated Faridpur soil

Incubation period....... ....*days*	0	3	7	15	30
NO_3—N in fresh soil .. ..*p.p.m.*	41.1	26.5	7.0	4.3	1.2
NO_3—N in incubated soil ....*p.p.m.*	60 1	57.6	55 1	53.1	48.1

little energy material in that soil, otherwise there would have been a lag in the accumulation of nitrate. On the other hand, results in table 1 and 2 show that in the same soil, when water-logged, more than 50 p.p.m. of nitrate was assimilated by the microörganisms in the same period, indicating that the soil had already contained a large excess of energy material. These two facts are apparently anomalous and require an explanation. It might be that a portion of the energy material, relatively less available in the dry soil, becomes easily available under the influence of water-logging, as a result either of hydrolysis or of greater solubility. In this connection it may be noted that to explain the rapid accumulation of nitrate in soils of Great Britain in late spring, Russell (11) assumed that "the winter frosts may cause a certain amount of physical disintegration of the organic matter and render some of it easily assimilable by the soil bacteria." Water-logging may bring about a similar change.

An experiment was next performed to observe the effect of the varying ratios between energy and nitrate on the assimilation of the latter by the microörga-

nisms. A culture solution[2] in 100-cc. lots was inoculated with 1-gm. portions of a soil previously water-logged; after incubation for 7 days at 33°C., total nitrogen was determined by the modified Davisson-Parson method. The results are given in table 7.

The results show that in the presence of sufficient energy material nitrate is rapidly assimilated by the microörganisms, from 30 to 50 parts of available carbon being necessary for assimilation of one part of nitric nitrogen, and that when the proportion of carbon is less than this amount a portion of nitrate is rapidly denitrified. Denitrification in soil must be an extremely slow process, for in none of the soils so far examined, not even in the incubated Faridpur soil in which the supply of energy is distinctly poor, did rapid denitrification similar to that observed in culture solution take place. In view of the fact that for microbial growth the conditions obtaining in culture solution are somewhat similar to those existing in water-logged soils, as far as the supply of air is concerned, it is permissible to advance the following hypothesis, on the basis of

TABLE 7

Effect of energy supply on the assimilation of nitrate

C N	NITROGEN, IN MGM.		
	At start	After incubation	Gain or loss
5:1	6 86	1.87	−4 99
10:1	6 86	2 52	−4 34
20:1	6 86	4.67	−2 19
30:1	6 86	4 78	−2 08
50:1	6 86	7 60	0.74
60:1	6 86	8 0	1 14

the above results, regarding the change of nitrate in water-logged soils in relation to the supply of energy: In soils having ratios of energy carbon to nitrate nitrogen greater than 30–50:1, nitrate will be rapidly assimilated; when the ratios are lower, a portion of the nitrate will survive in the soil and may denitrify only very slowly. The validity of this hypothesis was next tested by examining the incubated and the fresh Faridpur soil.

A proximate method of analysis of the soil organic matter has been suggested by Waksman and Stevens whereby 90–95 per cent of all the constituents of the organic matter of the soil can be accounted for in groups of definite chemical complexes. It has been found by this method of analysis that soil organic matter is made up of the following groups: 1, Fats, waxes, and resinous bodies; 2, carbohydrates, including various hemicelluloses and the cellulose-like bodies; 3, lignin-humus complex; 4, proteins. It will be seen that group 2 includes

[2] K_2HPO_4—1.0 gm., $MgSO_4 \cdot 7H_2O$—0.2 gm., NaCl—0.1 gm., $FeCl_3$—trace, KNO_3—0.5 gm., $CaCO_3$—10.0 gm., Water—1,000 cc., glucose—varying amounts to correspond to different C:N ratios.

carbohydrates, which readily serve as energy material for microörganisms. This group, therefore, represents the available energy content of soil. Thus, if from the total organic carbon of soil, the sum of carbon included in groups 1, 3, and 4 is deducted, the balance will represent energy carbon of soil. It is admitted that the values obtained in this way can be, at best, only a rough approximation of the true energy content, but for the purpose of comparison between two soils in terms of available energy these figures might be taken without serious error as representing the relative energy contents of the soils.

The results of the analysis of the organic matter of Faridpur soil, both the fresh and the incubated, are given in table 8.

By subtracting from the carbon found in the sulfuric acid residue, the carbon of the nitrogenous complex, assuming that the latter is present as protein and contains 50 per cent carbon, the percentage of the lignin-humus carbon in the soil is calculated as follows: Per cent of lignin-humus carbon in the fresh soil $= 0.373 - \frac{0.037 \times 6.25}{2}$, or 0.257. Similarly the per cent of lignin-humus carbon in the incubated soil is 0.307. Protein carbon in the fresh soil $= \frac{0.062 \times 6.25}{2}$, or 0.194, and protein carbon in the incubated soil $= \frac{0.056 \times 6.25}{2}$, or 0.175. Neglecting the carbon present as fat, etc., the energy carbon is obtained by subtracting the sum of protein carbon and lignin-humus carbon from the total organic carbon: Energy carbon in the fresh soil = 640 − 451, or 189 mgm. in 100 gm. of soil. The ratio of energy carbon to nitrate nitrogen = 189:0.56 (table 5), or 337.5:1; similarly, in the incubated soil it is 90:6, or 15:1.

TABLE 8

Analysis of soil organic matter

Results in per cent

	FRESH SOIL	INCUBATED SOIL
Organic carbon	0 64	0 572
Organic nitrogen	0.062	0.056
Carbon in sulfuric acid residue	0.373	0.435
Nitrogen in sulfuric acid residue	0 037	0.041

If the fresh soil is treated with 4 mgm. of nitrate nitrogen, as in the first two experiments (tables 1 and 2), the ratio becomes 189:4.56, or 41.2:1, which is almost equal to the limiting ratio (lying between 30:1 and 50:1). Hence the added nitate should be rapidly assimilated in this case, and actually it was so. When Faridpur soil is treated with 8 mgm. of nitrate nitrogen per 100 gm. of soil (table 1), the ratio becomes 189:8.56, or 22:1. Nitrate should not have been completely assimilated in this case. But it should be pointed out that this experiment was performed in the light, and consequently a portion of the ni-

trate was used up by the green matter for its growth. The narrow ratio 15:1 of the incubated soil clearly explains why nitrate suffered only a slight loss in this case.

EFFECT OF ADDED ORGANIC MATERIALS ON TRANSFORMATION OF NITRATE

The use of a large quantity of green manure in the rice fields and the thorough incorporation, during puddling, of the heavy crop of weeds formed on the dry soil necessitate a study of the effect of the added materials on the transformation of nitrate in water-logged soils. Three plant materials, viz., rice straw, water hyacinth (*Eichhornia Crassipes*), and "Kalai" (*Phaseolus Mungo*, var Roxb.), were powdered, and portions supplying equal amounts of carbon were thoroughly mixed with 50-gm. portions of Faridpur soil, which were then water-logged and treated with nitrate. Ammonia, nitrate, and total nitrogen were estimated at intervals. The results are given in table 9.

TABLE 9

Change of nitrate, ammonia, and total nitrogen in the presence of added material

Results of ammonia and nitrate expressed as p.p.m.; total nitrogen, as mgm.

PLANT MATERIALS	TIME IN DAYS									
	0		3		7		15		30	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N	NH_3-N	NO_3-N
Rice straw	2.2	45.4	5.8	0.3	0 3	0.3	0.3	0.3	0.6	0 3
Water hyacinth	2.2	45 4	21.6	0.3	3 2	0 6	55 7	0 1	94.1	1.0
Kalai	2.2	45.4	6.3	1.4	52.4	0.6	168.8	0.6	212 0	0.3
	Total N		Total N		Total N		Total N		Total N	
Rice straw	11.3		11.3		....		....		11 1	
Water hyacinth	23 6		23.6		....		....		21 4	
Kalai	21.7		21.7		....		....		17 1	

In every case nitrate quickly disappeared from the soil; the rate of disappearance was so rapid that even on the third day only a trace was present. The beneficial effect of green manure in water-logged soils thus appears to lie not only in supplying the nutrients to the crops but also in preventing the loss of nitrate by leaching. Waksman and Tenney (15) have shown that plant materials containing 1.7 per cent or more of N will undergo rapid decomposition without requiring the addition of extra nitrogen for that purpose. In view of this result, water hyacinth and Kalai should not have led to depletion of soil nitrate, as their own nitrogen (3.3 and 3.7 per cent respectively) is enough to meet the requirement of the microörganisms decomposing them. The rapid depletion of nitrate in this case suggests the possibility that microörganisms predominating under water-logged conditions may prefer nitrate to organic

nitrogen. As long as there was nitrate in the soil, the microörganisms may have attacked only the carbonaceous portions of the added materials for obtaining energy, the nitrogenous parts remaining largely unattacked during this period. After the nitrate was exhausted, the nitrogenous parts were then attacked, as shown by the rapid production of ammonia toward the latter part of the experiment. Soils treated with water hyacinth and Kalai lost a fair amount of nitrogen in a month. It is not understood how this loss was brought about. Similar loss of nitrogen has been noticed during the biological decomposition of organic materials of narrow C:N ratio (17, 18).

POT CULTURE EXPERIMENT

In order to get an idea of the magnitude of loss of nitrate from soil by bottom drainage, both in the presence and in the absence of a crop, a pot culture experiment was set up in which a known amount of nitrate was added to each of a

TABLE 10

Loss of nitrate from water-logged soil in drainage water

POT NUMBER*	MGM. OF NITRIC NITROGEN RECOVERED									
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	Total
1	....	....	....	. .	...	..	...	...	...	....
2	4.5	4 1	0 1	. .	...	..	..	...	...	8.7
3	6.2	13.9	0 1	....	...	..			. .	20 2
4	20.1	24 1	16.5	11.5	5 2	1.1	..		.	78 5
5	10 0	26 4	24.9	16 0	9 6	5 5	1 6	.	.	94 0
6	1 6	0 4	0 3	0 1	..	...	.		.	2 4

* Pot 1—unmanured but cropped; pots 2 and 3—manured and cropped; pots 4 and 5—manured but uncropped; pot 6—unmanured and uncropped.

number of pots and the leachate from each pot was collected and analyzed daily until it was found free from nitrate. The experimental details are as follows:

The experimental unit was a glazed earthenware pot (8 inches by 12 inches) with a single tubulure at the bottom of the side. Each was fitted with a delivery tube and mounted on a stand so as to allow a flask standing below it to receive drainage from the delivery tube. Six such pots were prepared, and a 2-inch layer of sand was put in each. A sample of Dacca Farm soil, sifted through a coarse sieve and thoroughly mixed, was fed into the pots 8 pounds at a time with gentle pressing until each pot contained 32 pounds of soil. The pots were then water-logged and allowed to rest for 3 months. Rice seedlings were transplanted into three of the pots, and water was drained through the delivery tubes daily. The soil in the pots was always kept water-logged by the periodic additions of distilled water. Before ear emergence, nitrate was applied as KNO_3 to four pots at the rate of 222 mgm. of nitric nitrogen per pot. The drainage was regulated in such a way that about 500 cc. of the drainage

water accumulated from each pot in 24 hours. After every 24 hours the receivers were changed and the nitrate leached out from each pot was determined. Even after nitrate had disappeared from the drainage water, analysis was continued for a few days more to ensure that nitrate had been completely washed out of the pots.

The results show that about 40 per cent of the added nitrate was recovered in the drainage water from the uncropped pots, but from the corresponding cropped pots the amount recovered was only 7 per cent.

SUMMARY

Experiments performed with a number of soils showed that nitrate is rapidly lost under water-logged conditions.

Detailed experiments with Dacca and Faridpur soils have shown that this loss is not due to the reduction of nitrate to ammonia; neither is there evidence to show that in soils under water-logged conditions all of the nitrate is denitrified.

An increased production of carbon dioxide and a rise in bacterial numbers occur in water-logged soil after addition of nitrate, showing that the added nitrate is assimilated by the microörganisms.

It has been suggested that, in soils having ratios of energy carbon to nitrate nitrogen greater than 30–50:1, nitrate will be rapidly assimilated by the microorganisms when such soils are water-logged; when the ratios are narrower, a portion of the nitrate will remain in the soils and may denitrify slowly.

In the presence of added organic materials, whether rich or poor in nitrogen, nitrate disappears very quickly.

A pot culture experiment showed that in the presence of a rice crop, little nitrate is lost in drainage water; in the absence of a crop, the loss is considerable.

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TOTAL NITROGEN AS A FACTOR INFLUENCING NITRATE ACCUMULATION IN SOILS[1]

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Although 50 pounds of nitrogen are required to produce an average acre grain crop and cultivated soils usually contain from 1000 to 4000 pounds of nitrogen in the surface foot, nitrogen is the nutritive element most frequently limiting plant growth in cultivated soils. These apparent contradictions arise from the fact that the soil's store of nitrogen is largely in a nonavailable form and becomes available rather slowly, particularly in soils that have been long under cultivation.

Because of the facts just stated, students of soil fertility have been much interested in the factors influencing the conversion of nonavailable nitrogen into an available form. Among the significant factors determining the ability of a soil to supply an adequate quantity of available nitrogen to a growing crop, the total quantity of combined nitrogen in the soil has been regarded as of paramount importance. Many data are on record indicating a direct relationship between the quantity of combined nitrogen in a soil and the ability of the soil to accumulate nitrate nitrogen during a limited period. There are, however, enough exceptions to this relationship to suggest that other factors may frequently be of equal or greater importance. In no instance, to the writer's knowledge, have data developed in such a study been submitted to statistical treatment to determine mathematically the significance of the apparent relationship. It is the purpose of this paper to present a limited study of this nature.

PRESENTATION OF DATA

Some years ago the writer (2) directed attention to a specific instance wherein the ability of a soil to accumulate nitrate nitrogen rapidly under laboratory conditions appeared to be more intimately associated with a certain small portion of its store of nitrogen than with the total quantity. In the accompanying discussion attention was directed to a suggestion made by Lawes, Gilbert, and Warrington (5) that the major portion of the store of nitrogen in a long-cultivated soil appears to be inert and that in such a soil the ability to supply

[1] Contribution No. 167, department of bacteriology, Kansas Agricultural Experiment Station.

a growing crop with adequate nitrogen depends, in part at least, upon the annual acquisition of a new supply of more easily nitrifiable nitrogen.

During the past few years a sufficiently large number of conditions somewhat similar to that noted above have been observed among soils studied to prove that the suggested explanation actually obtains in the field. The data that served as a basis for this conclusion are being presented elsewhere in the form of a detailed study of the rôle played by nitrogen in the production of "fertile" spots in 165 wheat fields (4). The term "fertile" as here used should apply literally only when a particular "fertile" sample is compared with its corresponding "nonfertile" sample. The "fertile" samples of soil were in all cases collected from small areas of soil such as previously pictured and described (3) as appearing in fields of small grain, and the "nonfertile" samples were collected from the area immediately surrounding the spots. Such spots frequently appear in fairly productive soils, hence the "nonfertile" of a pair of samples in one instance may have come from a much more highly productive soil than the "fertile" sample in another pair.

In the study referred to it was found that, in general (91 per cent of the comparisons), soil from the "fertile" spots possessed a more marked ability (on the average 3+ times) to accumulate nitrate nitrogen from its store of nitrogen than did the "nonfertile" sample collected only a few feet away. A statistical study of the data led to the conclusion that the more marked nitrate accumulating ability of the "fertile" soil was very definitely correlated with the presence of a small excess of total nitrogen over that present in the "nonfertile" soil. On the average this difference amounted to only 120 p.p.m. nitrogen. It was shown, though, that this 120 p.p.m. of nitrogen was capable of being converted into nitrate nitrogen more than 27 times as rapidly as was the other 1390 p.p.m. present in the "fertile" or in the corresponding "nonfertile" sample.

There are, no doubt, many soils that contain exactly the same quantity of combined nitrogen, but probably in no two soils, or two samples of the same soil, is this nitrogen present in the same forms. If, as has just been indicated, a small fraction of the nitrogen can play such an important rôle in the ability of the soil to accumulate nitrate nitrogen, and the combinations in which nitrogen exist in soils are so numerous, the question naturally arises: To what extent may such factors alter the influence of the total nitrogen as a factor controlling the nitrate accumulating abilities of soils?

In the investigation already referred to, the nitrate accumulating ability of 125 pairs ("fertile" and "nonfertile") of soils, or a total of 250 individual samples, was determined. These soils were also analyzed for total nitrogen, thus making possible a study of the relationship between the two factors. In no instance were soils selected on a basis of their probable nitrogen contents, yet they varied widely in this respect, being rather uniformly distributed over the range from 500 to 2000 p.p.m.

The details as to methods of collecting, handling, and analyzing the soils are being presented elsewhere (4) and need not be repeated here. Suffice it to

TABLE 1

Total nitrogen content and nitrate accumulating ability of 124 "nonfertile" soils

SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
1	330	44	43	1,310	99	85	1,530	110
2	380	33	44	1,320	151	86	1,540	156
3	550	134	45	1,320	17	87	1,550	181
4	560	71	46	1,320	59	88	1,560	111
5	600	71	47	1,330	145	89	1,560	96
6	610	36	48	1,330	102	90	1,570	55
7	740	83	49	1,340	139	91	1,570	80
8	760	54	50	1,350	78	92	1,570	47
9	770	71	51	1,350	193	93	1,590	114
10	960	52	52	1,360	39	94	1,590	185
11	960	142	53	1,360	138	95	1,600	88
12	1,010	87	54	1,370	88	96	1,600	194
13	1,030	75	55	1,370	34	97	1,610	142
14	1,030	70	56	1,370	198	98	1,630	78
15	1,030	91	57	1,380	101	99	1,660	164
16	1,030	140	58	1,380	57	100	1,660	181
17	1,050	136	59	1,380	133	101	1,670	92
18	1,080	66	60	1,390	154	102	1,670	43
19	1,080	95	61	1,400	342	103	1,690	270
20	1,100	132	62	1,400	128	104	1,700	75
21	1,110	132	63	1,400	141	105	1,700	59
22	1,120	97	64	1,410	138	106	1,710	221
23	1,120	149	65	1,410	98	107	1,720	181
24	1,130	44	66	1,410	81	108	1,730	147
25	1,130	61	67	1,420	152	109	1,800	83
26	1,140	99	68	1,430	195	110	1,810	85
27	1,150	131	69	1,430	52	111	1,810	195
28	1,160	77	70	1,440	44	112	1,820	101
29	1,180	149	71	1,440	177	113	1,830	101
30	1,200	41	72	1,440	189	114	1,840	111
31	1,220	67	73	1,450	79	115	1,860	232
32	1,220	85	74	1,460	120	116	1,860	128
33	1,230	63	75	1,460	73	117	1,880	60
34	1,240	151	76	1,470	91	118	1,890	280
35	1,240	102	77	1,470	92	119	1,890	208
36	1,250	75	78	1,470	193	120	2,060	158
37	1,270	61	79	1,470	72	121	2,110	182
38	1,270	45	80	1,490	219	122	2,150	164
39	1,270	184	81	1,500	71	123	2,160	166
40	1,290	248	82	1,500	176	124	2,300	215
41	1,290	89	83	1,510	59			
42	1,290	60	84	1,510	75			

Correlation coefficient 0.389±0.052.
Average nitrogen content 1390 p.p.m.
Average NO_3 content 116 p.p.m.

TABLE 2

Total nitrogen content and nitrate accumulating ability of 125 "fertile" soils

SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED	SAMPLE NUMBER	NITROGEN CONTENTS OF SOIL	NO_3 FORMED
	p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
1	360	−8	43	1,400	647	84	1,660	371
2	440	128	44	1,400	201	85	1,660	320
3	610	168	45	1,410	213	86	1,660	340
4	610	85	46	1,420	238	87	1,670	554
5	610	108	47	1,440	373	88	1,680	125
6	770	105	48	1,450	62	89	1,680	251
7	810	97	49	1,450	214	90	1,700	1,028
8	870	142	50	1,450	520	91	1,710	633
9	920	559	51	1,460	121	92	1,720	461
10	990	77	52	1,460	847	93	1,730	247
11	1,010	219	53	1,460	309	94	1,740	89
12	1,020	141	54	1,480	143	95	1,760	330
13	1,030	116	55	1,480	79	96	1,770	409
14	1,040	101	56	1,490	462	97	1,770	295
15	1,080	404	57	1,500	466	98	1,780	1,200
16	1,100	118	58	1,500	177	99	1,780	373
17	1,130	190	59	1,500	55	100	1,810	413
18	1,140	154	60	1,510	1,126	101	1,820	156*
19	1,150	524	61	1,510	758	102	1,820	515
20	1,190	71	62	1,520	1,086	103	1,840	136
21	1,200	445	63	1,530	324	104	1,870	782
22	1,250	64	64	1,540	193	105	1,870	254
23	1,250	632	65	1,540	274	106	1,870	1,418
24	1,260	316	66	1,550	189	107	1,880	223
25	1,270	147	67	1,550	161	108	1,880	611
26	1,280	52	68	1,560	414	109	1,890	430
27	1,290	283	69	1,570	368	110	1,910	98
28	1,300	348	70	1,570	201	111	1,920	302
29	1,300	507	71	1,580	112	112	1,950	546
30	1,300	188	72	1,580	779	113	1,950	537
31	1,310	267	73	1,580	382	114	1,950	875
32	1,350	610	74	1,580	889	115	1,970	741
33	1,350	80	75	1,590	111	116	1,980	471
34	1,360	778	76	1,590	234	117	2,020	249
35	1,360	255	77	1,610	101	118	2,020	349
36	1,380	200	78	1,610	597	119	2,080	546
37	1,380	69	79	1,620	250	120	2,140	724
38	1,380	986	80	1,630	1,571	121	2,180	305
39	1,380	96	81	1,630	171	122	2,210	1,081
40	1,380	244	82	1,630	538	123	2,210	447
41	1,390	380	83	1,650	186	124	2,310	769
42	1,400	212				125	2,310	332

Correlation coefficient 0.368±0.052.
Average nitrogen content 1510 p.p.m.
Average NO_3 content 375 p.p.m.

say that all samples were treated alike in that they were made up to optimum moisture content and incubated at room temperature 4 to 6 weeks, after which the nitrate (NO_3) content was determined. The initial NO_3 was deducted from that present after incubation, and the net gain thus obtained was taken as a measure of the nitrate accumulating ability of the sample.

The values obtained, by the afore-described procedure, from the "nonfertile" samples are presented in table 1, and those from the "fertile" samples, in table 2. In both tables the soils are arranged in the order of increasing nitrogen contents, hence the corresponding numbers in the two tables do not represent pairs of soils collected from the same field. In general, though, the paired samples will fall in the same group in table 3, where the data are summarized, the samples of similar nitrogen contents being grouped together and only the average nitrogen and nitrate values for these groups recorded.

TABLE 3

Average nitrogen content and nitrate accumulating ability of soils

RANGE IN NITROGEN CONTENT OF SOILS	"NONFERTILE" SERIES			"FERTILE" SERIES		
	Number of soils in group	Average nitrogen content	Average NO_3 formed	Number of soils in group	Average nitrogen content	Average NO_3 formed
p.p.m.		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*
0 to 1,000	11	656	72	10	699	146
1,001 to 1,200	19	1,098	98	11	1,099	226
1,201 to 1,400	33	1,321	114	23	1,336	329
1,401 to 1,600	33	1,498	117	32	1,513	371
1,601 to 1,800	13	1,688	134	23	1,689	454
1,801 to 2,000	10	1,849	150	17	1,893	501
Above 2,000	5	2,156	177	9	2,164	534
Average.		1,390	116		1,510	375

Correlation coefficient based on group averages: "nonfertile" series 0.990±0.012; "fertile" series 0.988±0.006.

DISCUSSION

A study of the data presented in table 3 alone could lead to but one conclusion, i.e., a very close and direct relationship exists between the nitrogen content of these soils and their ability to accumulate nitrates. A similar conclusion would be reached by a study of the oft quoted average values presented by Fraps (1). Furthermore, this conclusion apparently would be proved sound by a calculation of the correlation coefficients for the two sets of data recorded in table 3 and for the general average values recorded by Fraps, in that the almost perfect coefficients of 0.988±0.006, 0.990±0.012, and 0.995±0.003 are obtained respectively for the "fertile," "nonfertile," and Fraps' data. More nearly perfect correlation values could not be wished for.

Almost equally convincing relationships are sometimes obtained from a study of a limited number of more or less selected individual soils. For example,

among the few soils studied by Sievers and Holtz (7) there is no exception to the rule that, as the nitrogen content of the soil increases, its ability to accumulate nitrates increases, and the correlation coefficient calculated from these data, 0.921±0.057, approaches perfection.

On the other hand, when the data presented in tables 1 and 2 are studied in detail it becomes evident that there are a very large number of irregularities in the direct relationship between total nitrogen and nitrate accumulation. Similar marked irregularities are present in other instances where large numbers of soils, taken at random, have been studied; for example, in the data presented by Lipman, Burgess, and Klein (6).

The presence of so many marked exceptions in the relationship in any series could not help but influence materially the mathematical interpretation of data that is smoothed out by averaging results. In this instance it is found that the nearly perfect correlations obtained from the average values become almost negligible when calculated upon the individual samples. The 0.988±0.006 value for the "fertile" series becomes 0.368±0.052, and the 0.990±0.012 for the "nonfertile" series becomes 0.389±0.052; in both instances very little, if any, correlation is indicated. Since Fraps failed to record the data for the individual samples, similar comparisons cannot be made with his studies.

These correlation coefficients were so unexpectedly low in view of the close relationship shown by averaging data for the several groups that it seemed desirable to make a similar comparison with data obtained elsewhere. Because of the large number of soil samples involved, the data presented by Lipman, Burgess, and Klein (6) were chosen, and their humid and arid groups have been treated separately. Because of the limited number of humid samples, 47, they were divided into only five approximately equal groups on a basis of total nitrogen content and the average nitrogen content and nitrate accumulation for the various groups determined. By using the average values thus obtained as a basis for calculating the correlation between the two factors, the fairly significant value 0.727±0.142 is obtained. The corresponding value calculated on a basis of the individual samples is 0.479±0.073.

Similarly, the 140 arid soil samples were divided into groups on the basis of differences of 200 p.p.m. nitrogen up to 1600 p.p.m. and, because of the limited number, samples containing in excess of 1600 p.p.m. thrown into a single group, thereby making 9 groups each of which contained from 7 to 37 samples. When the average nitrogen and nitrate values of these groups were used as a basis for calculating the correlation coefficient, the value 0.686±0.119 was obtained. A similar calculation based upon the 140 individual samples gave a coefficient of only 0.190±0.037. Other comparisons with comparable results have been made. It would thus appear that the relationships found to exist between the nitrogen content and the nitrate accumulating ability of these Kansas soils are of the same order as similar relationships based upon data reported by other investigators.

The object in calling attention to the facts presented in the preceding pages has not been to discredit the value of total nitrogen as a factor limiting the

accumulation of nitrates in soils, but rather to suggest the possibility of arriving at erroneous conclusions when data of this nature are loosely interpreted by averaging values, as has been rather freely done in the past.

The fact should not be lost sight of that these studies have been based upon a relatively short incubation period. It is difficult to see how the total quantity of nitrogen could help but be a major limiting factor in nitrate accumulation when the time factor is eliminated.

From a practical point of view, the ideal rate of nitrate formation should be just sufficient to prevent nitrogen from becoming the limiting factor in plant growth. In the studies already referred to, it is shown that, in the "nonfertile" soils here discussed, nitrogen was a major limiting factor in the growth of the wheat plant. The presence of the 120 p.p.m. additional nitrogen in the fertile soil largely eliminated this condition, not because of the additional nitrogen alone, but because it was in such a form that it could be more readily changed into an available condition. So long as the quantities of easily nitrifiable nitrogen vary in soils it cannot be expected that a perfect correlation will exist between nitrogen content and nitrate accumulation, at least during a limited incubation period. The greater the variation in the quantities of such forms of nitrogen, the less marked will be the direct relationship between the two factors. Apparently such variations are sufficient in Kansas soil that, coupled with other factors, the influence of total nitrogen upon nitrate accumulation may be almost completely overshadowed during relatively short periods of time.

SUMMARY

When the nitrogen content and the nitrate accumulating abilities of a large number of soils are determined and the data thus obtained are grouped on a basis of the nitrogen content of the soils and averaged, an almost perfect direct relationship may appear to exist between the total nitrogen content and the nitrate accumulating ability. On the other hand, if the original data are used as a basis for calculating the coefficient of correlation, the relationship between the two factors may be found to be very slight, or even nil.

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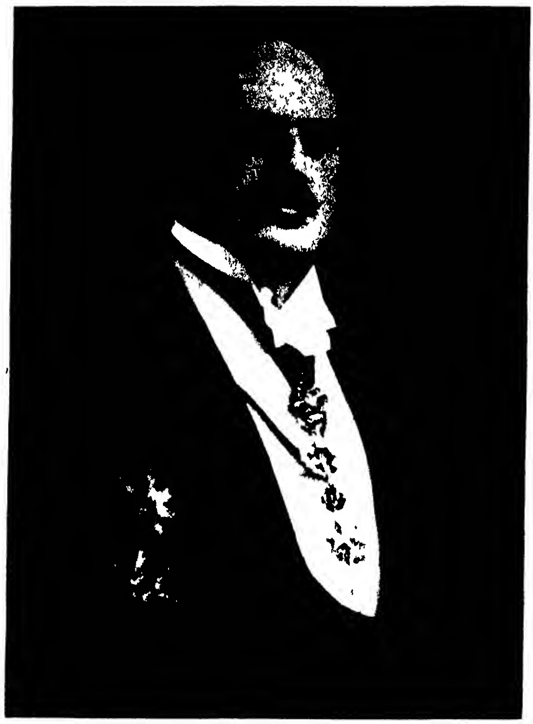

Julius Stoklasa

Julius Stoklasa[1]
1860-1936

One of the greatest agricultural scientists and one of the most interesting personalities of our day, Dr. Julius Stoklasa, died in Prague, April 4.

Stoklasa began his professional studies in Tetschen in 1873. After two years he entered the Agricultural Institute in Vienna. Later he studied in Leipzig, under Oswald and Pfeffer, and in the Pasteur Institute in Paris, where he began his work in soil biology. It is at these institutions that the young student learned, in the words of Professor Bornemann-Eisenwach, "to understand and comprehend the longing for life, and above all the life of plants."

Stoklasa's research activities extended over a wide field. His first publication, the thesis for his doctorate at Leipzig, "Über die physiologischen Eigenschaften der wasserlöslichen Verbindung der Phosphorsäure in den Superphosphaten," pointed to the future trend of his life work. As a foundation for his work in plant physiology, Stoklasa undertook a study of soil processes. He became particularly interested in the rôle in plant nutrition of certain specific elements; namely, arsenic, aluminum, and iodine. He also investigated various changes which take place in the growing plant, and soon became a pioneer in the field of biochemistry, especially in the study of enzymes. In his studies on the transformation of phosphorus, his attention was called to the importance of bacteria, while his biochemical investigation of the soil led him directly to the study of soil bacteriology, whereby he contributed to making this an independent science. Thus he penetrated further and further into the nature of plant life in his search for the source from which organisms draw their life energy. In time, these studies led to the discovery of the influence of radioactivity on the growth and movement of matter in organisms.

Because of their great number—more than 400—it is impossible here to enumerate the scientific contributions of Stoklasa, which cover an enormous field of research. The commemoration volume published by P. Parey in Berlin in 1928 in honor of Stoklasa's seventieth birthday, gives a comprehensive account of his work. To be sure, this account is now incomplete, since Stoklasa continued to spend the last ten years of his life in diligent research. Among his extensive publications, mention should be made of "Der Biochemische Kreislauf des Phosphat-ions im Boden," "Biophysikalische und biochemische Durchforschung des Bodens," and his work on the smoke damage problem and on the cycle of aluminum in nature. His last investigations dealt with the biology of radium, of which only the first volume has so far appeared.

[1] Prepared by Dr. E. G. Doerell, formerly one of Professor Stoklasa's assistants.

In many instances, Stoklasa developed new methods and propounded theories to answer both general and specific questions. Whether or not one agreed personally with these theories, it must be admitted that they stimulated further scientific investigation. As the alchemists are considered the founders and prophets of modern chemistry, in the same light Stoklasa may be regarded as one of the last great alchemists. The near or distant future will probably verify many of his hypotheses.

In spite of his specialized researches, Stoklasa was anything but one-sided. He was the typical prewar *Grand Seigneur* in appearance and character, a man of the world who embraced the universe in his studies. His life work was devoted to an investigation of the relations between life processes and living systems. It is not by chance, therefore, that in his works he always returned to the soil as the source of life.

Stoklasa was a prominent organizer. He is numbered among the founders of such institutions as the Czechoslovakian Academy of Agriculture and the Technical Museum. He also served as president of the Third Commission of the International Society of Soil Science. He was a brilliant orator who, by his eloquence, captivated not only professional scientists but also practical farmers.

The death of Stoklasa brings to a close a life rich in results and in numerous honors. The spirit of Stoklasa, whose life work was to fathom the "Spirit of the Soil," will continue, through his work, to influence the development of soil science.

SEASONAL CHANGES OF THE MOISTURE CONTENT OF SOLAGA SOIL OF PALESTINE AND THEIR INFLUENCE ON VEGETATION

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The deficiency of soil moisture is the chief factor which determines the presence and development of plant life under the soil conditions of Palestine. With the exception of that on irrigated land the vegetation corresponds to the season as well as to the total rainfall during the year and the amount of moisture preserved in the soil during the dry season.

In the Beer Sheba district, for instance, which is distinguished by low rainfall and high permeability of loess soil, the cultivation methods of the native population aim toward the accumulation of water in the depths of the soil and its preservation from evaporation.

These methods empirically found and others newly introduced into Palestinian practice may be accepted only after detailed study of the water balance in the soils. With this in view, the moisture content and the amount of soluble salts were recorded under field conditions in the different depths of loamy Solaga soil for two years.

THE PROPERTIES OF SOLAGA SOIL

The Solaga soil, the water retaining conditions of which were studied, belongs to the common type of heavy soils of the coastal plain. The study of the soil profile indicates the presence of two layers as follows:

The upper layer, which reaches a depth of 40 cm., is distinguished by a good structure. This layer is composed of aggregates of particles and is perforated with channels, formed by plant roots. Being continually cultivated, it shows better physical properties than the subsoil layers of the soil profile.

The lower layer, below a depth of 40 cm., appears as a structureless heavy soil which absorbs much water and swells extensively.

The study of the nature of exchangeable bases in this soil has shown that the upper layer contains more exchangeable Ca and less exchangeable Na. than the lower layer. The average annual rainfall in the belt of this soil is about 500 mm.

ANNUAL CHANGES IN WATER CONTENT OF DIFFERENT LAYERS OF SOLAGA SOIL

Samples of soil from different layers of the most typical locality were collected from a certain plot which had not been cultivated. In order to get a

distribution of the soil moisture, unaffected by vegetation, we invariably destroyed the wild plants springing up during the rainy season. The collected samples were examined, and the moisture content as well as the amount of soluble salts in the soil was recorded.

The graphical interpretation of these records, in figure 1, shows the character of the distribution of the soil moisture in different depths during different seasons of the year. The months when the soil samples were collected are represented on the abscissa, and the moisture of soil examined, expressed in percentage, on the ordinate. The four curves thus obtained correspond to the four layers of the soil *within 25 cm. of one another* and show the annual *courses* of the moisture content in different layers of Solaga soil.

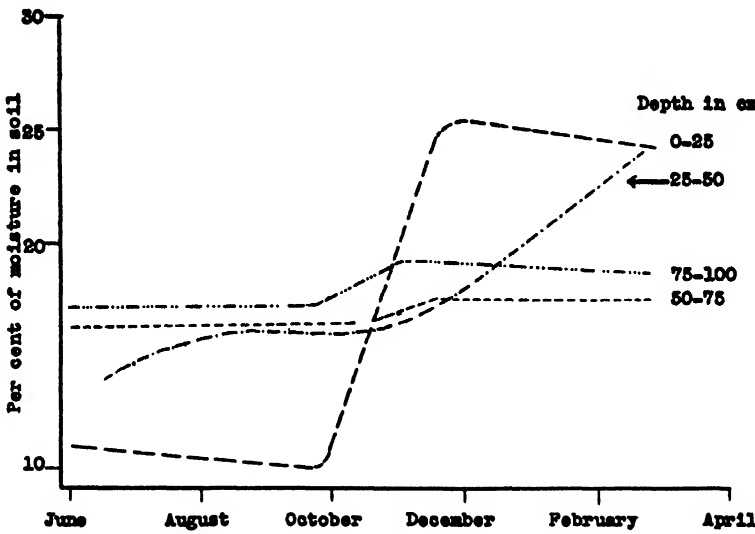

FIG. 1. ANNUAL COURSES OF THE MOISTURE CONTENT OF DIFFERENT LAYERS OF SOLAGA SOIL

The curve for the 0–25 cm. depth indicates that in the month of June there is about 11 per cent of moisture in the soil. This figure gradually decreases until by October only 9.5 per cent remains, representing the lowest water content found in this type of soil. June–October is the dry season and the hottest time of the year in Palestine. The value of night dew in the formation of soil moisture has not yet been studied here. In any case, the dew does not increase the moisture content of the surface layer, and the heat of the following day removes the last traces of non-hygroscopic water of the soil. In November the rainy season begins, and the curve of moisture shows a sharp rise at this time, continually increasing until December, when the maximum is reached. It subsequently slowly decreases until the end of the dry season. The course of the curve illustrates well the yearly changes in water content of the surface soil in Palestine.

The curve for the second layer, i.e., the 25–50 cm. depth, is different from the one just described. The lowest figure, which is 13 per cent, found in the month of June, shows that the minimum of moisture in the second layer is greater than the minimum in the first (0–25 cm.) for the same season. The course of this curve shows a constant increase until the month of March, when the moisture content of the soil reaches its maximum of 23 per cent. In contrast to the 0–25 cm. layer, the moisture content of which continually decreases during the June–October period, the moisture of the 25–50 cm. layer continually increases during the same interval. This increase of water content in the 25–50 cm. depth can be explained by the process of distillation. Soil moisture from the surface layer, continually heated, becomes vapor, which in turn condenses in the lower layer. Lebedev (2) found that in cases where the water content of the soil reaches the maximum hygroscopicity, the relative humidity of the soil air reaches 100 per cent. According to his data, the soil of southern Russia even at a depth of 5–10 cm., is saturated with water vapor. As a rule, the temperature of the soil decreases with the depth, and the pressure of the water vapor declines with it. Possessing higher pressure, the water vapor of the upper layer moves to the lower layer, where it condenses. The second part of the curve belongs to the rainy season. It shows that the rains increase the moisture content of the soil to a lesser degree than in the previous layer. This difference can be explained by the physicochemical properties of the soil, which cause it to swell greatly and thus prevent the passage of the water to the subsoil.

The curve for the third layer, i.e., 50–75 cm., has an entirely different character. The moisture in this depth remains within the limits of 15.8 to 17.0 per cent throughout the year. The influence of the rainy season is manifested here only by a slight increase of moisture in the soil (about 1.0 per cent).

The curve for the 75–100 cm. layer generally resembles the course of the 50–75 cm. curve, the figures, however, being relatively higher.

SEASONAL CHANGES IN WATER-SOLUBLE SALTS IN THE SOIL

The general content of water-soluble salts is a distinctive factor in determining soil types as well as conditions of plant growth. The seasonal changes in temperature and in amount of rainfall influence the amount and concentration of soluble salts in the soil at different depths.

The quantities of soluble salts in the plot were determined, and the concentration of the salts in the liquid phase of the soil was calculated for the dry and wet seasons.

The first curve (0–25 cm.) in figure 2 shows that for the June–October period the concentration of soluble salts is similar to that of soils in arid regions whereas during the November–March season it is similar to that of soils in humid regions. The same properties are observed with the three other curves, but less distinctly. The curves showing changes in concentrations of salts in the 50–75 and 75–100 cm. depths during the year were compared with the

corresponding, approximately constant, moisture distribution curves, and thus the *rhythmic up and down movements of the soluble salts in the soil occurring in the course of the year were demonstrated.*

MOLECULAR FORCES OF SOIL AND WATER RETENTION

The fact that the amount of moisture at a shallow depth (50–75 cm.) remains constant during the very dry and hot season of the year when the soil is strongly insolated can be explained by the existence of strong molecular forces which keep the water on the surface of the soil particles. The character of water absorptions in the soil can be seen in table 1, in which moisture of air-dry soil, hygroscopicity according to Mitscherlich, and moisture in the soil

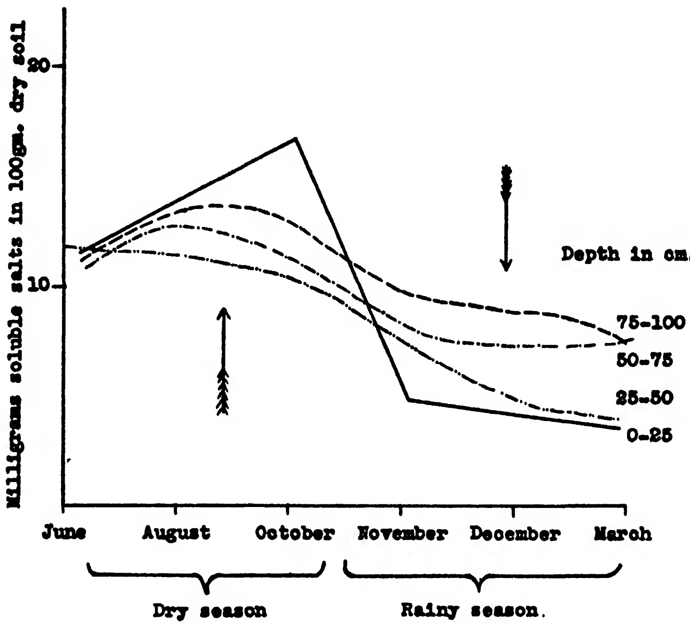

FIG. 2. CONCENTRATIONS OF SOLUBLE SALTS IN DIFFERENT LAYERS OF SOLAGA SOIL
Arrows show the up and down movement of the soluble salts

equivalent to the coefficient of Mitscherlich, as well as fluctuations of soil moisture during the year, were recorded. The proximity of the figures for hygroscopicity according to Mitscherlich to those for the moisture content in the 50–75 cm. layer is especially evident for the dry season. These peculiar properties of the 50–75 cm. soil layer can be explained by the low temperature of the soil, the higher concentration of soluble salts, and the greater proportion of exchangeable Na in the absorbing complex of this layer. These three factors work in unison for the lowering of the water vapor pressure and the preservation of water in the soil. The property of heavy hydration of the soil, the absorbing complex of which is saturated with monovalent bases, has been ascertained by several workers (7). The second and third factors are conse-

quences of the semiarid climatic conditions which are predominant in these soils. We thus arrive at the conclusion that *the greater part of this water is retained by surface forces and is therefore hardly available to the common plants.* According to the classification of Lebedev, this water is partly "hygroscopic" and partly "membrane" water.

DISTRIBUTION OF MOISTURE IN THE SOIL AND SEASONAL CHANGES OF VEGETATION

The wild vegetation of Palestine is distinguished by the change of plant associations in accordance with different climatic and soil conditions during the various seasons of the year. These changes are brought about in the following way (fig. 3):

Most of the tuberous and bulbous plants develop, flourish, and bear fruit from the beginning of October until February.

The annual plants appear in November or December and grow until the end of May, gradually associating themselves with the previous flora of tuberous and bulbous plants. From January to May the annual plants predominate.

TABLE 1

Moisture content of Solaga soil

DEPTH OF SOIL	MOISTURE IN AIR-DRY SOIL	HYGROSCOPICITY ACCORDING TO MITSCHERLICH	MOISTURE IN SOIL EQUIVALENT TO COEFFICIENT OF MITSCHERLICH	FLUCTUATIONS IN MOISTURE CONTENT OF SOIL DURING THE YEAR
cm.	*per cent*		*per cent*	*per cent*
0–25	9 55	16.29	14.0	9.25–25.1
25–50	9 24	15.56	13.5	13.0 –23.2
50–75	9.25	16.02	13.9	15.7 –17.0
75–100	9.74	16.90	14.5	16.3 –19.1

The perennial plants. The beginning of the season of perennial plants coincides with the period of vegetation of the annual plants and continues until the end of September.

In October this vegetation cycle begins again.

The plant associations, mentioned are composed of species adapted to the different soil climatic conditions. Their presence and changes are dependent chiefly on the distribution of soil moisture during the year.

The appearance of the tuberous and bulbous plant formations is possible only in the *humid soil conditions which, as has been shown, exist only during the October–March interval in the 0-25 cm. depth.* The percolation of rain water to the 50 cm. depth during the following months favors the development of the vegetation of annuals, the roots of which reach that depth. The dry season of the June–October period hinders the physiological activity of the bulbous and tuberous plants and prevents the existence of the annual plants, the roots of which take their water from the 0–25 cm. and 25–50 cm. depths. Since our observations proved that during this same season the moisture of the 50–100

cm. depth remains constant throughout the year, the existence of the perennial plants and of those annuals the roots of which reach that depth is possible.

Two sets of field crops, each containing different species of plants, are known in the agricultural practice of Palestine. They are the *summer* and *winter crops*, the growth of which correlates with the aforementioned changes in soil moisture conditions and their corresponding wild vegetation.

The character of the moisture retained by Solaga soil raised the question whether this moisture would, as a rule, be available to the common plant.

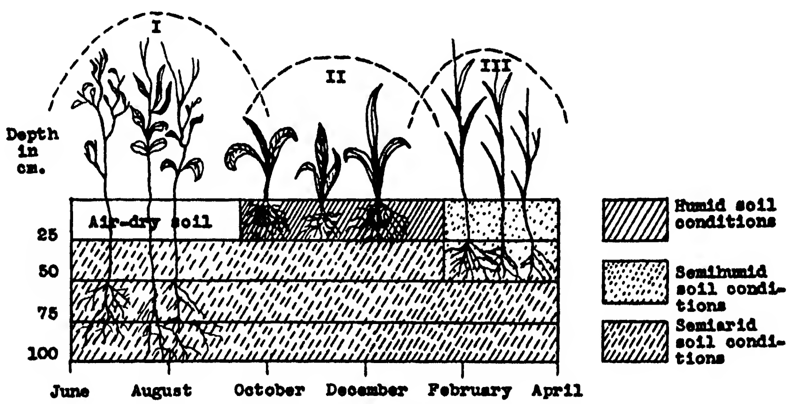

FIG. 3. SEASONAL CHANGES OF THE WILD VEGETATION OF PALESTINE

I—Season of predominant perennial and annual plants with roots longer than 50 cm.
II—Season of predominant bulbous plants.
III—Season of predominant annual plants.

TABLE 2

"Wilting points" of different layers of Solaga soil

DEPTH OF SOIL	WATER ABSORBED BY 100 GM. OF SOIL IN ATMOSPHERE SATURATED WITH VAPOR	WILTING POINT CALCULATED ACCORDING TO FORMULA OF BRIGGS	WATER IN SOIL EQUIVALENT TO WILTING POINT OF BRIGGS	WATER IN SOIL EQUIVALENT TO WILTING POINT ACCORDING TO BOGDANOV
cm.	*gm.*		*per cent*	*per cent*
0–25	18.4	26.6	21.0	19.1
25–50	18.5	26.7	21.1	18.5
50–75	18.3	26.5	20 9	18.5
75–100	17.5	25.3	20.2	19.5

Table 2, which shows the wilting points for the different layers of Solaga soil, throws more light upon the subject. These figures are obtained by the calculation of the amount of moisture absorbed by air-dry soil in the atmosphere saturated with water vapors according to the formula of Briggs (1), and of the doubled hygroscopicity of air-dry soil according to Bogdanov. Only in the 0–25 cm. layer during the October-March period and in the 25–50 cm. layer at the end of the wet season, when the soil reaches its maximum water content, does the amount of moisture in the soil exceed or even reach the wilting point.

In the lower layers of the soil, i.e., 50–100 cm., in depth, the maximum moisture content is much lower than the wilting point and approaches the coefficient of Mitscherlich's hygroscopicity. Since not only hygroscopic water, but also a "distinct part of the capillary water" (3), is generally unavailable to the plants, this water scarcity phenomenon renders the problem of the plant water supply in the heavy soils of Palestine different from the same problems in humid climates.

XEROPHYTIC CHARACTER OF PALESTINE'S VEGETATION

The drought resistance of the plants has been interpreted by Fitting and Maximov as a physicochemical property (5): "The high concentration of cell sap allows the plants to grow in the dry atmosphere and under conditions of insufficient soil moisture. . . . Only through the high osmotic pressure can the xerophytic plant in the wilting state develop an important water absorbing capacity. At the same time the increased concentration of the sap causes dehydration of the protoplasm and membranes, thereby decreasing the loss of water by the plant."

In Palestine the October-March season is the germination period of Gramineae. As already stated, there is a high moisture content, which is propitious

TABLE 3

Ash content of straw

VARIETY OF PLANT	EUROPE	UNITED STATES	PALESTINE
	per cent	*per cent*	*per cent*
Wheat	4.7	4 2	10.6
Barley	5.0	5.7	9.7
Maize	...	3.4	13.6

to root development, in the upper layer of soil (25 cm.) during the rainy season. Later, however, when the roots reach a depth of 50 cm., they enter into layers having a water content little above or even lower than the wilting point. It was natural, therefore, to find in the composition of cell sap some aid to plant growth. The ash content of the plants may, in such a case, give some idea of the concentration of cell sap.

The composition of straw ash in Palestine and in other lands is presented in table 3. The ash content of the straw of wheat and barley is high in comparison with that of European and American crops. This high salt content may play an important part in the establishment of a high osmotic pressure of cell sap, which in turn renders the problem of obtaining moisture from the soil less difficult and enhances the loss of water in the exceedingly dry air of Palestine.

Here, in the unfavorable air and soil conditions, the xerophytic character of the local corn with an ash content which reaches 13.6 per cent is more pronounced than that of any other grain. In Palestine the maize develops during the months of April-August, when the water content of the surface layer of soil

reaches 10.5–9.5 per cent, a state of air dryness. For this period the moisture, as shown in table 1, was preserved only at a depth of 50–100 cm. The necessary moisture for the corn plant, therefore, can be obtained in the following ways: by sending the roots to a depth of 100 cm. or more and through the high osmotic pressure of the cell sap, which permits the absorption of soil moisture, which is lower than the wilting point for plants of humid climates.

SUMMARY

The changes in the dry and wet periods of the year in Palestine influence the moisture content of only the superficial layer of Solaga soil (0–50 cm.).

The greatest changes in moisture content were observed in the 0–25 cm. layer, from a minimum in October (air-dry soil) to a maximum in December (about 24 per cent).

The moisture content of the 50–100 cm. depth remains about constant during the year, with a slight increase during the wet season (about 1 per cent).

The changes of plant associations are closely connected with the soil moisture distribution during the year at different depths. *The bulbous and tuberous plants* vegetate during the short period of high moisture content of the 25 cm. depth during the months of October to March; *the annual plants* the roots of which penetrate not more than to a 50 cm. depth grow from November until May; *the perennial plants* obtain their water mostly from a 50–100 cm layer depth, where the moisture remains about constant during the year.

With few exceptions (the temperate humid conditions during the wet season at a 25 cm. depth) the normal amount of moisture in Solaga soil is lower than the moisture content equivalent to the wilting point of plants of humid climate. Important elements of the Palestine flora as well as local varieties of agricultural plants possess a more or less pronounced xerophytic character which helps them to exist and develop in the insufficient soil moisture conditions.

The high ash content of some local grains proves their xerophytic character. The high osmotic pressure of their cell sap permits the absorption of the water imbedded in the soil.

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A GREEN MANURE FERTILIZER STUDY ON NORFOLK SAND[1]

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This report gives results of an experiment which is part of a general study of green manure and fertilizer problems planned to develop means of improving sandy soils of the South. The results were obtained at the South Carolina Agricultural Branch Experiment Station (the Sandhill Experiment Station), near Columbia, S. C., which is located in the Sandhill belt of the Southeast.

The sandhill area of the Southeast is shown in figure 1. That portion which lies in South Carolina varies in width from 10 to 30 miles, and comprises approximately 10 per cent of the area of the state. The soils are principally of the Norfolk and related series. The sandhill phase of Norfolk sand on which the experiment was made is of a coarse and an open texture. The native cover, following deforestation of the long-leaf pine, is principally dwarf oak, sedge, and other plants capable of growing on soil low in fertility and subject to excessive leaching. The cultivated areas are used for the production of cotton and corn; more recently peaches, grapes, dewberries, and asparagus are being grown.

REVIEW OF LITERATURE

The various symposia which have been held in recent years (20, 21, 22) have focused attention upon the pertinent relations of organic matter to soils and crops. Excellent résumés of the literature have been made by Waksman (27) on the nature of organic matter, by Thorne (25) on its relation to crop production, and by Salter (14) on the composition as related to its effectiveness.

The factors which influence the decomposition of organic matter in the soil have been investigated by Thom and Smith (24), Thom and Humfeld (23), Waksman (26), and others.

The effect of the carbon-nitrogen ratio of various organic materials and of the soil itself upon the availability of nitrogen in the soil has been investigated

[1] A contribution from the Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the South Carolina Experiment Station, cooperating, at the Sandhill Experiment Station, Columbia, S C Credit is due Dr. J. J. Skinner, of the Bureau of Plant Industry, for advice in developing plans of the studies and for guidance in prosecuting the work, and acknowledgment is made to the Office of Forage Crops and Diseases, Bureau of Plant Industry, for their aid in the management of plats.

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by Brown and Allison (5), Doryland (7), Blair and Prince (4), Jensen (11), Salter (15), Conrad (6), and many others. The Norfolk soils are classified by Anderson and Byers (2) as yellow podzols. Waksman and Hutchings (28) point out that the organic matter of podzols is characterized by a high cellulose and hemicellulose and a low protein content which produces a ratio, for the soils mentioned, of 22.5 to 41.7. The ratio for the Norfolk sand used in this experiment is approximately 26. These soils contain charcoal due to the prevalent practice of "burning over" during the early spring season; this

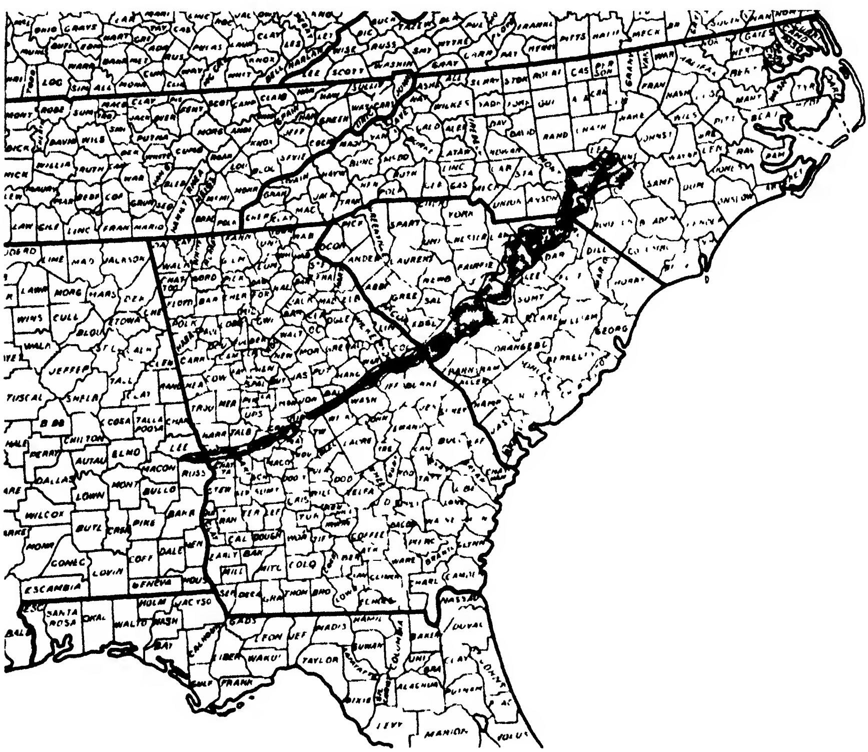

Fig. 1. Sandhill Section of North Carolina, South Carolina, and Georgia Lying between the Piedmont and Atlantic Coastal Plains Belts

Shaded section is the sandhill section (From U. S. Dept. Agr. Tech. Bul. 452, 1934)

contributes to the wide ratio. Heyward and Barnette (10) and Greene (9) have found this practice to be beneficial.

The lighter Norfolk soils of North Carolina are rated by Williams et al. (29) with a value of 1 to 7, the best soils being rated at 10. It is considered here that the results obtained on the Norfolk sand differ from those on the heavier types in degree rather than in general nature. The fertilizer studies of Skinner (16), Skinner and Buie (17), and Skinner et al. (18) have demonstrated the need for complete fertilizers for the production of corn and cotton,

with emphasis placed on the need for nitrogen. Bell (3) has found that the decomposition of some legumes depresses the nitrate-nitrogen content of Norfolk sand. Cowpeas and manure, however, were found to increase the nitrate content of the soil.

Funchess (8) states that the low crop yields of the South are due more to a deficiency of available plant food than to any single factor, and that "the need for nitrogen is greatest from a relative standpoint." The need for nitrogen on Norfolk sand is outstanding.

DESCRIPTION OF EXPERIMENT

The area of Norfolk sand used in this experiment was cleared in 1919.[4] It was cropped to corn during the years 1920, 1921, and 1927; the last season mentioned was the first year it was under the supervision of the State of South Carolina. Cotton was grown from 1922 to 1926, inclusive. The corn was fertilized with 400 of a 4–8–4 fertilizer[5] and side-dressed with 100 pounds of nitrate of soda per acre. The cotton received 800 pounds of fertilizer of a like formula and the same amount of nitrate of soda as for the corn.

Twenty-four plats (1/20 acre), arranged in six tiers of four each and replicated once, were used in a rotation of legumes, corn, and cotton. The tiers are paired for the sake of comparison. Legumes, i.e., soybeans, velvet beans, and cowpeas, were grown on one tier with 400 pounds of a 6–8–4[6] fertilizer and on the second tier with a like application of a 2–8–4 fertilizer. An additional plat of cowpeas, to be followed by a winter cover crop of rye and vetch, completed each of the two tiers. The hay was removed from the tier receiving the 6–8–4 fertilizer, and the stubble was turned; the full crop was turned as a green manure in early September of each year on the tier receiving a 2–8–4 fertilizer. The same fertilizer ratios were maintained on the stubble and green manuring plats for the corn and cotton grown successively in the rotation. The corn received an application of 400 pounds per acre; the cotton, 800 pounds. The year 1934 marked the end of the second round of the rotation.

METHODS

Soil samples were taken semi-annually during the experiment. These were analyzed for total carbon by the wet oxidation method of Adams (1), and for total nitrogen by the Gunning-Hibbard method, as given in the Official Methods of the Association of Official Agricultural Chemists. The pH values were determined according to the hydrogen-electrode procedure of Snyder (19).

[4] History of area furnished by Mr. J. A. Riley, Superintendent, Sandhill Experiment Station, Columbia, South Carolina.

[5] NH_3-P_2O_5-K_2O.

[6] The fertilizer ratio is $N:P_2O_5:K_2O$. One-third of the nitrogen was derived from nitrate of soda, one-third from sulfate of ammonia, and one-third from cottonseed meal; the phosphoric acid was from 16 per cent superphosphate; and the potash, from sulfate of potash.

YIELDS DATA

The average yields for all crops are given in table 1 for the first round (1929–1931) and the second round (1932–1934) of the rotation, as well as for the 6 years. The yield of hay in plats where the vegetation was turned under was obtained by cutting and weighing several areas 5 feet by 5 feet and returning the vegetation. The growing corn and cotton, the indicator crops, are shown in plate 1. The 6–8–4 fertilizer and stubble system has produced larger yields of all crops than has the 2–8–4 and green manure. Average

TABLE 1

Average yields, per acre, of crops grown on legume-stubble plats with 6–8–4 fertilizer and on green-manure plats with 2–8–4 fertilizer, on Norfolk sand: 1929–1931; 1932–1934; 1929–1934

SUMMER CROP AND WINTER MANAGEMENT	STUBBLE TURNED PLUS 6–8–4			GREEN MANURES TURNED PLUS 2–8–4*		
	Green Hay	Corn	Seed Cotton	Green Hay	Corn	Seed Cotton
	tons	*bushels*	*pounds*	*tons*	*bushels*	*pounds*
Soybeans, fallow:						
Ave. 1929–31	2.15	10.77	437	1.64	8.71	290
" 1932–34	2.04	11.51	688	1.53	8.83	375
" 1929–34	2.10	11.14	563	1.59	8.77	333
Velvet beans, fallow:						
Ave. 1929–31	3.68	9.49	368	2 66	8 17	227
" 1932–34	3 22	10.72	567	2.40	8.46	306
" 1929–34	3.45	10.11	468	2.53	8.32	267
Cowpeas, fallow:						
Ave. 1929–31	2.09	10.15	370	1 63	7.10	221
" 1932–34	2.49	10.94	627	2.20	8.94	322
" 1929–34	2.29	10.55	499	1.92	8.02	272
Cowpeas, rye, and vetch:						
Ave. 1929–31	2.45	11.53	564	1.98	9.22	371
" 1932–34	3.06	15.12	915	2.67	11.06	472
" 1929–34	2.76	13.33	740	2.33	10.14	422

* Legumes and corn received 400 pounds per acre; cotton, 800 pounds.

yields of seed cotton for 6 years from the former system show increases over the latter as follows: soybeans, 230 pounds; velvet beans, 201 pounds; cowpeas, 227 pounds; and cowpeas, rye, and vetch, 318 pounds. The yields of corn are consistently greater than those of the 2–8–4 and green manure plats but are not in proportion to those of cotton.

The cash values of the crops grown under the 6–8–4 and stubble system are considerably greater than those under the other, because of increased yields of corn and cotton and of the hay, which is handled as a cash crop. When a uniform basis for the cost of materials and crops produced is used, the follow-

ing increased values, per acre per year, are obtained: soybeans, $5.61; velvet beans, $7.29; cowpeas, $5.60; and cowpeas, rye, and vetch, $7.82. The high value of velvet beans in the rotation is due principally to the hay produced rather than to an unusual increase in the production of corn and cotton. An additional value should be attached to the winter cover of rye and vetch due to conservation or maintenance of nitrogen, as shown in table 2.

The pH of the plats decreased from 5.5 to 4.8 during the first round of the rotation. An application of 300 pounds of hydrated lime was made each spring of the second round to the two tiers of plats on which legumes were to be grown. This allowed the full effect to be exerted on the legumes. The pH changed from 4.8 to 5.8. The data for the second round gives the effect of lime upon the legumes for 3 years, upon corn for 2 years, and upon cotton for 1 year.

TABLE 2

Carbon and nitrogen relationships in Norfolk sand, with legume stubble turned and crops grown with a 6–8–4 fertilizer, as compared with legumes turned as green manures and crops grown with a 2–8–4 fertilizer, in a rotation of legumes, corn, and cotton

SUMMER CROP AND WINTER MANAGEMENT	STUBBLE TURNED PLUS 6–8–4 FERTILIZER						GREEN MANURES TURNED PLUS 2–8–4 FERTILIZER					
	Total C		Total N		C/N		Total C		Total N		C/N	
	1928	1934	1928	1934	1928	1934	1928	1934	1928	1934	1928	1934
	per cent	*per cent*	*per cent*	*per cent*			*per cent*	*per cent*	*per cent*	*per cent*		
Soybeans, fallow	0 54	0 40	0 020	0 018	27 0	22 2	0 51	0 36	0 020	0.017	25 5	21 2
Velvet beans, fallow	0 48	0.38	0 020	0 018	24 0	21 1	0 50	0 38	0 020	0 018	25 0	21.1
Cowpeas, fallow	0 52	0 38	0 020	0 019	26 0	20 0	0 51	0 38	0 020	0 017	25 5	22.3
Cowpeas, rye, and vetch	0 54	0.43	0 019	0 020	26 8	21.5	0 50	0.42	0.019	0 020	26 3	22.1

The average yeilds of soybeans and velvet beans were decreased during the second round, while those of cowpeas, for both systems as well as for fallow and the winter cover, were increased. Corn showed a slight but consistent increase during the second round for both systems. The gains for the cotton during the second round over those of the first averaged 263 pounds of seed cotton per acre on the 6–8–4 and stubble plats, while the increase for the other system was 84 pounds. The stubble and 6–8–4 combination is superior to the green manure and 2–8–4 management, except in the case of corn grown on the cowpea-winter fallow plats. Not only was the greatest increase in yields of both crops obtained on the cowpea-rye-vetch plats but also the greatest total yields. If there was any effect of lime on these crops during the second round, it was entirely overshadowed by the differences due to the combined fertilizer treatment and crop management.

CARBON-NITROGEN DATA

Charcoal in this soil, due to the practice of "burning over" already discussed, undoubtedly contributes to the wide carbon-nitrogen ratio. Any change in

the ratio during the course of the experiment, however, should be due to the particular treatment. Comparisons should, therefore, be valid. The type of vegetation and the coarse texture of the soil, which allows excessive leaching, give rise to organic matter of a wide ratio and low in amount. The soil appears to be analogous to a sand culture to which material of a wide carbon-nitrogen ratio has been added. The management supplying the greatest quantity of nitrogen available to the indicator crops should show increased yields. The data indicate that the extra 4 per cent of nitrogen applied to the stubble plats supplies a greater quantity of available nitrogen than does the other system of management.

The carbon and nitrogen data are given in table 2. Leighty and Shorey (12), Anderson and Byers (2), and McLean (13) have shown that the variable

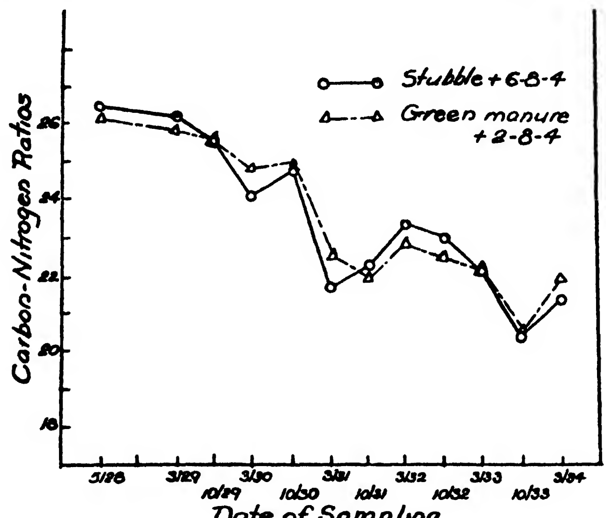

FIG. 2. CHANGE IN CARBON-NITROGEN RATIOS FOR AVERAGE OF ALL PLATS OF THE 6-8-4 AND STUBBLE SERIES VS. 2-8-4 AND GREEN MANURING SERIES, FOR ORIGINAL SAMPLES OF MAY, 1928, TO SAMPLES OF MARCH, 1934

carbon-nitrogen ratio of soils makes unreliable the use of a factor to convert organic carbon to organic matter. For this reason, and because of the presence of charcoal in the soil, the term "carbon" is used except where the term "organic matter" is used in the broadest sense.

A very appreciable loss of carbon has occurred on each of the 48 plats, irrespective of the fertilizer treatment and management. These represent from 16 to 27 per cent of the total carbon present at the beginning of the experiment. The use of rye and vetch has resulted in a smaller loss under both systems of management. There are losses of nitrogen on all plats left fallow during the winter season, the losses being slightly greater where the crops were turned as green manures. Plats on which cowpeas were followed by a winter cover show a slight gain in nitrogen for both systems of management.

The stubble and 6–8–4 system is slightly superior to the green manure and 2–8–4 system in regard to the conservation of organic carbon and nitrogen when winter fallow is practiced. When a winter cover is used these two systems are of equal value.

There has been a considerable decrease in the carbon-nitrogen ratio for each of the 48 plats. Figure 2 depicts the change in the carbon-nitrogen ratio obtained as an average of all 24 plats on which the 6–8–4 fertilizer and stubble management was used, as compared with the same number of 2–8–4 and green manure plats, irrespective of the crops grown. The data give a direct comparison of the effect of each of the systems upon the ratio, for the years 1928 to 1934, and show that both systems affect the ratio equally. It is thought that the increased yields of corn and cotton during the second round of the rotation

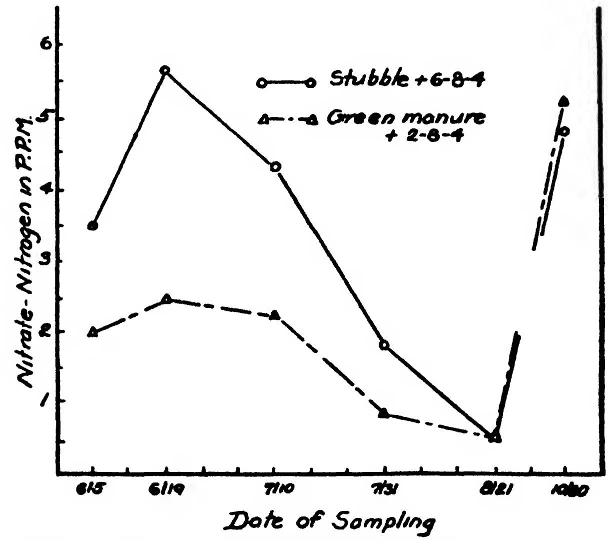

FIG. 3. NITRATE NITROGEN IN SOIL OF 6–8–4 AND STUBBLE PLATS VS. 2–8–4 AND GREEN MANURE PLATS DURING 1933

Last half of fertilizer applied June 15; stubble and green manures turned August 18

are associated, in a general way, with the decreased ratios. The reduction should result in a greater supply of nitrogen available for plant growth. The rate at which the ratio is decreasing indicates that some years will probably elapse before an optimum ration will be established.

NITRATE NITROGEN

Determinations of the nitrate nitrogen throughout the summer season of each year, for which tables are not given, show a low average content. Figure 3 shows the average nitrate nitrogen content for the season of 1933 for the 6–8–4 combination versus the 2–8–4 combination, calculated in the same way as for the carbon-nitrogen ratios. The 1933 season was chosen because leaching was not excessive. Excessive rainfall would minimize these differences.

The generally increased supply of available nitrogen due to the use of the 6–8–4 fertilizer has produced larger average yields of all crops.

RESIDUAL CARBON AND YIELDS

Figure 4 presents graphically the relations between the carbon content of the plats, at the end of the second round of the rotation, and average yields of corn and cotton for the 6 years. Tabular data are not given. Data for the plats are plotted separately rather than as averages of two replications.

The relations of yields to the residual carbon contents of the plats are masked by the influence of the nitrogen in the fertilizer and by the effect of

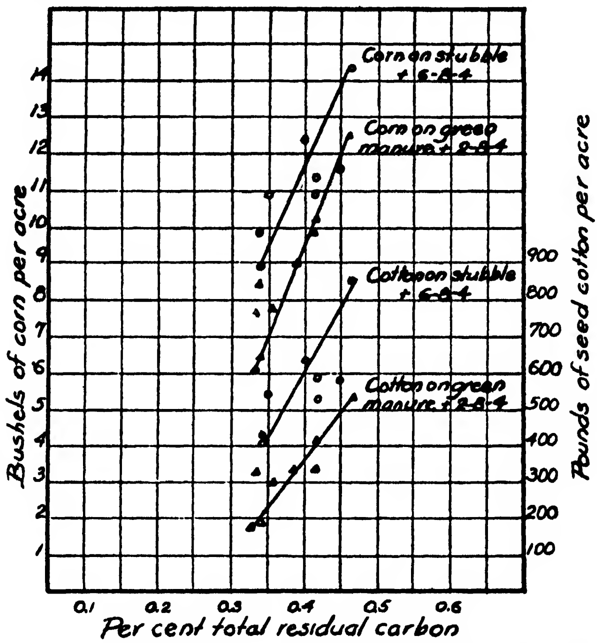

FIG. 4. RELATION OF YIELDS OF CORN AND COTTON TO RESIDUAL TOTAL CARBON CONTENT OF PLATS, WHEN GROWN ON LEGUME STUBBLE AND SUPPLEMENTED WITH 6–8–4 FERTILIZER, AS COMPARED WITH LEGUMES TURNED AS GREEN MANURE AND FERTILIZED WITH 2–8–4

the winter cover crop. There is a loose correlation between carbon and yields; it is somewhat better for the green manure and 2–8–4 system than for the stubble and 6–8–4.

SUMMARY

A comparison has been made of legumes stubble with the full crops of the legumes turned as green manures, when used in a rotation with corn and cotton. Crops grown on stubble plats were fertilized with materials forming a 6–8–4 ratio; those grown where legumes were turned received a 2–8–4 fertilizer. From data obtained on Norfolk sand, the following conclusions are drawn:

A 6-8-4 fertilizer applied to crops grown where the stubble of soybeans, velvet beans, and cowpeas had been turned produced larger yields than where the 2-8-4 fertilizer supplemented the green manures, all plats being left fallow during the winter.

Rye and vetch used as a winter cover following cowpea stubble, and also where cowpeas were turned as green manure, produced greater yields than on plats allowed to lie fallow during the winter. The higher nitrogen fertilizer-cowpea stubble-winter cover combination produced the largest yields.

The use of the stubble and 6-8-4 combination results in a greater conservation of carbon and nitrogen than the green manure and 2-8-4 combination. The two systems are practically of equal efficiency in the case where a winter cover follows both cowpea stubble and cowpeas turned as a green manure.

Yields of corn and cotton were but loosely associated with the total carbon content of the soil at the end of the second round of the rotation. This correlation was less apparent when increased quantities of nitrogen were applied in the form of commercial fertilizers.

There has been a considerable reduction in the carbon-nitrogen ratio of the soil. This is thought to be associated with an increase in the availability of the nitrogen present.

Yields were influenced more by the nitrogen of the fertilizer than by the management of the legumes. The winter cover was apparently more effective in influencing yields than was the legume green manure or legume stubble.

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PLATE 1

FIG. 1. Cotton in green manure fertilizer experiment 1933. Left, cotton following cowpea stubble turned, 800 pounds per acre of 6–8–4 fertilizer; center row, no fertilizer; right, cotton following cowpeas turned as green manure, 800 pounds per acre of 2–8–4 fertilizer.

FIG. 2. Corn in green manure fertilizer experiment 1933. Left, corn following cowpea stubble turned, 400 pounds per acre of 6–8–4 fertilizer; center row, no fertilizer; right, corn following cowpeas turned as green manure, 400 pounds per acre of 2–8–4 fertilizer.

FIG. 1

FIG. 2

PHOSPHORUS NUTRITION OF CITRUS AND THE BENEFICIAL EFFECT OF ALUMINUM

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Lemon cuttings grew readily in shallow nonaerated solution cultures, but orange or grapefruit cuttings offered considerable difficulty. Omission of the phosphate from the solution for various periods of time proved beneficial, but the use of aluminum in the absence of phosphate gave the best growth.

It is possible that there is a precipitating action of aluminum with phosphorus, not only without, but within the plant and an essential need for aluminum in citrus. This paper deals with the phosphorus nutrition of citrus and its relation to aluminum.

The methods employed in growing the cuttings, in preparing the material for analysis, and in determining the total phosphorus have previously been described (2)

PREVIOUS INVESTIGATIONS

The investigations on phosphorus and aluminum have become so numerous that only the papers pertinent to the present problem will be discussed. The literature on the phosphorus relation in citrus has previously been briefly summarized (2). The essential nature of aluminum for plant growth was indicated in studies conducted by Sommer (8).

Aluminum in concentrations as low as 1 p.p.m. in solutions lacking phosphate was found by Ligon and Pierre (3) to be definitely injurious to plant growth. Aluminum depresses growth, according to Line (4), by removing phosphate or by raising the acidity but not as a result of aluminum toxicity.

By using the citrate as the source of aluminum, McLean and Gilbert (5) demonstrated that aluminum is toxic even in solutions with very low acidity (pH 6+), and that from 3 to 13 p.p.m. of aluminum constitute a stimulating dose for the plants used, toxicity appearing at about 16 p.p.m. Sideris (7) found that pineapple plants in culture solutions endured concentrations as high as 25 p.p.m. without injury.

EXPERIMENTAL

In preliminary studies, rooted lemon leafy-twig cuttings were used in 2-quart Mason jars as the containers for aerated and nonaerated culture solutions. The solutions of some cultures contained 105 p.p.m. phosphate added

as KH_2PO_4; in others the phosphate was omitted; and in still others 0, 5, 10, 15, 20, 25, and 30 p.p.m. of aluminum were added as the citrate to the phosphate-free solution. The culture solution lacking phosphate had the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

The cuttings were grown from January 29, 1930, to December 23, 1931. At the conclusion of the experiment the cuttings showed a beneficial effect of phosphate on the growth of the tops. A too-prolonged absence of phosphorus at the higher concentrations of aluminum affected the tops in that the leaves occurred at considerable intervals on long shoots. The presence of aluminum improved the health of the root system and retarded the growth of the tops. The roots, when supplied with aluminum, were healthy white in color and grew deep down into the culture solution regardless of the factor of aeration.

In preliminary experiments, lemon seedlings also were used to test the effectiveness of aluminum on growth. The plants were grown in a nonaerated culture solution of the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	PO_4	Cl	Fe	Mn	B
7	185	54	0	225	216	105	10	0.1	6 2	0.5

plus 0.1 p.p.m. of minor elements such as I, Br, Ti, Zn, NH_4, and Sr. Calcium citrate was added to the solution in some jars and calcium aluminate in others as a source of calcium.

The absence of calcium brought about injury to the roots, and the tops then wilted and died (pl. 1, fig. 1). The plants grown in a solution supplied with calcium as the citrate were able to produce some growth in the tops and roots, but the root growth was accompanied by injury. The use of calcium aluminate brought about a beneficial effect not only in the roots but also in the tops.

In experiments with large citrus cuttings grown for approximately one year or more, a study was made of the phosphorus content in relation to the general growth response obtained when a given culture solution was used.

Attention was first given to the phosphorus content in various portions of lemon cuttings grown in culture solutions of maintained pH. Lisbon and Eureka lemon cuttings were grown from February 24 to December 7, 1933, in nonaerated culture solutions in some of which the pH was maintained at definite levels (pH 4.5) by means of dilute nitric acid. In some solutions 105 p.p.m. phosphate was continually present; in others it was either absent at all times or was present (105 p.p.m.) for only a few days at intervals of several weeks. The culture solution lacking phosphate had the following composition (p.p.m.):

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

Various concentrations of aluminum as the nitrate or citrate were added to some of the culture solutions.

Plate 1, figure 2, shows the growth, in the absence of aluminum, of cuttings in phosphate-free cultures and in cultures in which 105 p.p.m. phosphate was present for a few days at intervals of several weeks. The tops and roots were in a healthy condition when phosphate was present only at intervals. Although very little growth was made when phosphate was continually absent, the roots remained in good condition.

In the presence of aluminum the top growth is poorer and the root growth better in the continued absence of phosphate (pl. 1, fig. *3A*) than when phosphate is present occasionally in the culture solution (pl. 1, fig. *3B*). Comparison of the growth made by the cuttings in the cultures without phosphate (pl. 1, fig. *2A* and *3A*) indicates that aluminum has greatly improved the root system when phosphate was deficient.

Comparison of the growth made by the cuttings in the cultures containing phosphate for a few days at intervals of several weeks (pl. 1, fig. *2B* and *3B*) indicates that the aluminum stimulated the growth of the root system but at the apparent expense of the top growth.

Gericke (1) has directed attention to the beneficial effect to plant growth of the temporary depletion of some of the essential elements in the soil. The results for citrus have indicated that when phosphorus is temporarily absent from the culture solution the presence of aluminum is beneficial to the root growth. The concentrations of phosphate in the soil solution fluctuate from time to time, and the periods of minimum phosphate concentration may be considered of advantage to the plants in that the concentrations of other elements have an opportunity to increase.

Pierre and Stuart (6) found that the beneficial action of large phosphate additions to acid soils is partly due to a reaction of aluminum and phosphate within the plant and not entirely to the precipitation of aluminum in the soil solution.

In the absence of aluminum in the nonaerated culture solution, there were beneficial effects on the growth of the root system of Eureka lemon leafy-twig cuttings when phosphate was omitted and a poor type of root development when 105 p.p.m. phosphate was present. As shown in plate 1, figure 4B, both root systems were finely divided and dark in color, especially that grown in the high phosphate solution. The phosphate deficiency leaf symptoms were just beginning to make their appearance in the plants grown in the solutions lacking phosphate.

Other Eureka lemon cultures (pl. 1, fig. *4A*) were grown in solutions containing 105 p.p.m. phosphate for a few days at intervals of several weeks and contained aluminum during the omission of the phosphate.

The presence of aluminum in the solution most of the time, with phosphate supplied for only a few days at long intervals, is particularly beneficial to the root system. When aluminum is present, the roots are white, coarse, and

TABLE 1

Phosphorus content, as per cent of dry matter, of various portions of lemon cuttings grown in culture solutions of maintained pH

LAB. NO.	DURATION OF THE EXPERIMENT (1933)	CULTURE SOLUTION	P CONTENT OF		
			Leaves	Shoots	Roots
		(*Lisbon lemon*)			
1	Feb. 24 to Dec. 7	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 always absent (pl. 1, fig. 3*A*)	0.0488 0.0494*	0.0291 0.0275*	0.0600 0.0750*
2	Feb. 24 to Dec. 7	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 3*B*)	0.0599 0.0650*	0.0369 0.0375*	0.1250 0.1625*
		(*Eureka lemon*)			
3	April 11 to Dec. 26	pH 4.5 with HNO_3; Al always absent; 105 p.p.m. PO_4 always present (pl. 1, fig. 4*B*)	0.2500	0.2688	0.3125
4	April 11 to Dec. 26	pH 4.5 with HNO_3; Al and PO_4 always absent (pl. 1, fig. 4*B*)	0.0875	0.0431	0.0800
5	April 11 to Dec. 26	pH 4.5 with HNO_3; 10 p.p.m. Al added as the citrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 4*A*)	0.1000	0.0438	0.2000
6	April 11 to Dec. 26	pH 4.5 with HNO_3; 20 p.p.m. Al added as the citrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks (pl. 1, fig. 4*A*)	0.0938	0.0525	0.3625
7	April 11 to Dec. 26	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.1125	0.0675	0.2500
8	April 11 to Dec. 26	pH 4.5 with HNO_3; 20 p.p.m. Al added as the nitrate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.1219	0.0538	0.2781
		(*Lisbon lemon*)			
9	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; Al always absent; 105 p.p.m. PO_4 always present.	0.1750	0.1800	0.7000
10	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; 15 p.p.m. Al added as the sulfate; 105 p.p.m. PO_4 present for a few days at intervals of several weeks	0.0600	0.0350	0.2000
11	Mar. 4 to Dec. 23	pH 4.5 with H_2SO_4; Al always absent; 105 p.p.m. PO_4 present for a few days at intervals of several weeks.	0.0613	0.0438	0.1188

* Repetition experiments.

healthier in appearance than when aluminum is absent. At a concentration of 20 p.p.m. aluminum, whether as the citrate or the nitrate, the growth of both tops and roots is retarded. Later, these effects upon the roots will be given more detailed consideration.

In table 1 are given the percentages of phosphorus in the dry matter of the various portions of lemon cuttings. The cultures were grown in phosphate-free solutions of the following composition:

Na	K	Mg	Ca	NO_3	SO_4	Cl	Fe	Mn	B
7	185	54	159	789	216	10	0.1	6.2	0.5

To this solution were added various concentrations of aluminum in the form of the citrate, nitrate, or sulfate. Phosphate, when present, was added as potassium acid phosphate.

When 105 p.p.m. phosphate was always present in nonaerated culture solutions with lemon cuttings, the roots became gelatinous and the leaves light-veined. Table 1, numbers 3 and 9, indicate the high phosphorus concentration in leaves of such cuttings.

Excellent vegetative growth of the tops was obtained when the phosphorus content of the dry matter of the leaves was between 0.06 and 0.12 per cent. No definite evidence was obtained that the addition of aluminum to the culture solution increased the percentage of phosphorus in the dry matter of the leaves.

A concentration of 15 to 20 p.p.m. of aluminum was rather high for the production of the greatest growth. pH 4.5 is rather close to the minimum pH value that the roots will withstand. The great metabolic activity of lemon cuttings (in comparison with that of orange) permits lemon roots to change more readily the pH of the culture solution toward the more favorable higher pH values. The nature of the acid and similarly that of the alkali used in regulating the pH values of the culture solutions for the growth of citrus cuttings have an effect on the growth produced. In the presence of considerable sulfate, it is more difficult to maintain a green color in the leaves, although an increased amount of iron is of benefit.

When lemon cuttings are grown in the presence of aluminum and in the absence of phosphorus, burning of leaves may occur because of a deficiency of phosphorus. On account of the reduced top growth in the presence of considerable aluminum and in the absence of phosphorus, the leaves remain attached for a longer time than when aluminum is also absent.

In the early stages of growth the roots of Lisbon lemon cuttings in nonaerated culture solution containing at all times 105 p.p.m. phosphate, are light colored and the laterals short. Later the roots become extremely dark-colored and gelatinous. In the absence of phosphate in the culture solution until deficiency symptoms are evident in the tops, the roots have longer laterals than when 105 p.p.m. phosphate is continually present. As the phosphate deficiency becomes more acute, the roots become dark colored but re-

main alive. In the continued absence of phosphate and in the continued presence of aluminum, the plants have few but long sturdy roots which remain in a healthy condition longer than roots grown without aluminum. The presence of aluminum in the solution most of the time, with phosphate supplied for only a few days at long intervals, maintains this healthy condition of the root system in solution cultures.

The beneficial action of aluminum may be due to the slow but steady supply of phosphorus obtained from the reutilization of phosphates precipitated by the aluminum in or on the roots. The presence of aluminum in the culture solution when phosphate continually is omitted is of assistance, not only in retarding the growth of the top and thereby slowing up the injury to the leaves, but in increasing the health of the root system. This latter condition will later be seen as being of assistance in promoting the recovery of phosphorus-starved cuttings once phosphate is supplied in the culture solution.

The healthy condition of root tips of citrus cuttings grown in solution culture may be due to the essential need of aluminum for root cap production and possibly for the growth of the root tips themselves. Evidence was obtained that the production of root caps was increased by the presence of aluminum in the culture solution. The lemon cuttings, the roots of which are shown in plate 2, figure 1, were obtained from solution cultures containing aluminum. It is seen that many of the root tips are covered by root caps. These were not evident in roots of cuttings grown without aluminum in the culture solution.

The recovery of lemon cuttings from a severe phosphorus deficiency in which all except the youngest leaves had fallen was next studied in relation to the presence or absence of aluminum in the culture solution. The cultures were conducted from February 19 to December 22, 1933, and the pH of the culture solutions was maintained at 4.5 by means of dilute nitric acid. The cuttings grown with aluminum in the culture solution possessed larger root systems than those grown without it. On and after August 30, 1933, 105 p.p.m. phosphate was added for a few days at intervals of several weeks to the phosphate-deficient cultures.

The growth of lateral buds is related to the supply of phosphorus. When aluminum was present in the phosphate-deficient culture solution, the lateral buds started to grow within a few days after phosphate was supplied to the culture solution. The growth of lateral buds in the cultures that received no aluminum eventually starts and is tardily followed by root growth, but the recovery of top and root growth is markedly more rapid when aluminum is supplied. In other experiments of a similar kind it was found that recovery in the tops parallels the state of health of the roots.

It is of interest that the young terminal leaves are better able to resist the injuries that result from a phosphorus deficiency than are more mature leaves. It is known that immature leaves contain higher percentages of phosphorus than mature ones.

Lemon cuttings were grown from February 19 to June 7, 1933, in a culture

solution containing 20 p.p.m. of aluminum but no phosphate. The pH of the culture solution was not controlled and ranged between pH 5 and 7, which was more favorable to growth than pH 4.5. On June 7 the cuttings had lost all except the immature terminal leaves, and no lateral shoots had developed. From June 7 to November 9, 1933, 105 p.p.m. of phosphate was used in the

TABLE 2

Phosphorus content, as per cent of dry matter, of various portions of Lisbon lemon cuttings grown in culture solution and recovering from phosphate deficiency

LAB. NO.	DURATION OF THE EXPERIMENT	CULTURE SOLUTION	P CONTENT OF		
			Leaves	Shoots	Roots
1	July 14, 1932, to December 1, 1933	pH 7.6 on Feb. 19, 1933; Al and PO_4 always absent	0.0406	0.0213	......
2	February 19 to November 9, 1933	pH 7 to 8; Al always absent; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0.1125	0 0925	0 1625
3	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al and PO_4 always absent	0 0438	0 0244	0 0506
4	February 19 to December 22, 1933	pH 4.5 with HNO_3; PO_4 always absent; 10 p.p.m. Al added as the nitrate	0.0325	0.0238	0 0456
5	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al always absent; PO_4 absent until August 30, 1933, and subsequently present for a few days at intervals of several weeks	0.0450	0 0250	0 0800
6	February 19 to December 22, 1933	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 absent until August 30, 1933, and subsequently present for a few days at intervals of several weeks	0 0563	0 0263	0.3031
7	February 19 to December 22, 1933	pH 4.5 with HNO_3; Al always absent; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0 0475	0.0275	0.1225
8	February 19 to December 22, 1933	pH 4.5 with HNO_3; 10 p.p.m. Al added as the nitrate; PO_4 absent until June 7, 1933, and subsequently present for a few days at intervals of several weeks	0.0613	0.0319	0.2500

culture solution for a few days at intervals of several weeks and aluminum was present when phosphate was absent from the culture solution.

At the conclusion of the experiment a multiplicity of new lateral shots was produced after phosphate was supplied, which shows that the lateral buds were still alive. Growth was very rapid, and the leaves were somewhat chlorotic.

The leaves were broad and not in a reflexed position at the petiole junction with the leaf blade. The roots showed vigor and were in excellent condition.

The percentages of phosphorus in the dry matter of the various portions of phosphate-starved and recovered lemon cuttings are shown in table 2. Extreme phosphorus deficiency symptoms were accompanied by low percentages of phosphorus in the leaves (numbers 1, 3, and 4 in table 2). The percentages of phosphorus found in the leaves of recovering plants varied from those typical (number 2 in table 2) of continually healthy lemon plants to those typical (numbers 5 and 7 in table 2) of phosphorus-starved plants. The percentage values indicate that the cuttings were thoroughly depleted of phosphorus at the time the phosphorus supply was resumed. The production of new growth in the recovering plants prevented any rapid build-up of the phosphorus concentrations in the leaves during the initial stages of recovery. The percentages of phosphorus in these recovering cuttings gives some conception of the minimum phosphorus concentrations necessary in citrus.

The percentages of phosphorus shown in table 2 are to be considered as being in equilibrium only for a particular phase of the recovery process. Data in this regard are given in table 3. All leaves analyzed were considered to be mature, and from the data obtained it is seen that the percentage of phosphorus in the original lemon leaves is subject to considerable change, depending on the phosphate content of the culture solution. In many cases of phosphate deficiency, there is a smaller percentage of phosphorus in the original than in the subsequently produced mature leaves. When the culture solution contains an abundance of phosphate, the original lemon leaves still contain the smaller percentages, although all of the percentages are on a higher level. Whenever 105 p.p.m. of phosphate was continually present in nonaerated culture solution, the root system eventually was injured, whereas with aeration such roots were dwarfed.

Excellent growth of pomelo leafy-twig cuttings as scions on Eureka lemon leafy-twig cuttings as stocks was obtained in culture solutions in which 105 p.p.m. of phosphate was present for only a few days at intervals of several weeks and the aluminum was present, if at all, only when phosphate was absent. The growth of the cuttings when aluminum was not used in the culture solution was considerably less than that produced during the same growth interval when aluminum was present.

It is possible that beneficial effects that occur in the absence of phosphate may be due, not alone to its absence, but to the appearance or solubility in the culture solution of traces of elements ordinarily removed for the most part from the solution when phosphate is present. To contribute information in this direction, the following experiment was devised. One set of lemon cuttings was grown in a nonaerated culture solution (pH 4.5) containing 105 p.p.m. phosphate. Another set of cultures was grown for 2 days in a culture solution containing 105 p.p.m. phosphate, then for 2 days in a similar solution lacking phosphate, and then again for 2 days in the original (fresh) solution

TABLE 3

Phosphorus content, as a per cent of dry matter, of various portions of lemon cuttings grown in culture solutions and injured by or recovering from phosphate deficiency

DURATION OF THE EXPERIMENT	CULTURE SOLUTION	P CONTENT OF		
		Leaves	Shoots	Roots
(*Lisbon lemon*)				
July 19, 1932, to January 5, 1933	PO_4 always absent; 10 p.p.m. Al added as the citrate	0.0736 0.0500† 0.0569*	0.0280	....
July 19, 1932, to January 5, 1933	PO_4 always absent; 20 p.p.m. Al added as the citrate	0.0563 0.0602*	0.0389	....
July 19, 1932, to January 5, 1933	105 p.p.m. PO_4 always present	0.2063	......	......
July 19, 1932, to January 5, 1933	105 p.p.m. PO_4 always present	0 2100 0.1350*	0.3000	....
July 15, 1932, to December 1, 1933	pH 7.3 on February 19, 1933; Al and PO_4 always absent	0 0363	0.0250	......
July 19, 1932, to December 1, 1933	pH 7.8 on February 19, 1933; 10 p.p.m. Al added as the citrate; PO_4 always absent	0.0405	0 0500	......
July 28, 1932, to December 1, 1933	pH 8 on February 19, 1933; 20 p.p.m. Al added as the citrate; PO_4 always absent	0.0373 0.0398†	0 0188 0 0206†	....
July 28, 1932, to December 10, 1933	Composite of cuttings from 2 culture solutions; 10 p.p.m. Al added as the citrate, pH 7.8 on February 19, 1933; 20 p.p.m. Al added as the citrate, pH 6.2 on February 19, 1933; PO_4 absent until February 19, 1933, and subsequently 105 p.p.m. PO_4 present for a few days at intervals of several weeks; Al present when PO_4 absent	0.1100	0.0675	0.1844
July 14, 1932, to January 5, 1933	PO_4 absent	0 0500	......	......
July 14, 1932, to January 5, 1933	PO_4 absent	0 0619 0.0500*	0.0350	....
October 21, 1932, to July 5, 1933	PO_4 absent	0.0500 0.0450*	0.0250	....
October 21, 1932, to July 5, 1933	105 p.p.m. PO_4 always present	0 1625 0.0865*	0 1850	....
(*Villafranca lemon*)				
February 27 to July 9, 1934	PO_4 absent	0.0675 0.0494*	. ...	0.1025 ......

* Original leaves of cuttings.

† Repetition experiment.

containing 105 p.p.m. phosphate. During the absence of phosphate in the solution, the impurities in the salts from which the stock solutions are prepared had an opportunity to remain more soluble and to affect the root or top growth. Since no change in the growth of the root system was observed, the salt impurities apparently had no effect, possibly because of insufficient concentration.

In two other sets of cultures like the preceding ones, similar changes of culture solution were made, except that 10 p.p.m. of aluminum was present when phosphate was omitted. The roots of these cultures showed a definite response to the added aluminum. A different interval from 2 days or a different pH range of the culture solution would alter somewhat the degree of response obtained.

The beneficial effect of the presence of aluminum during the interval in which phosphate was omitted in nonaerated cultures in which rooted Valencia orange cuttings were grown is shown in plate 2, figure 2. The cultures were grown from March 18 to September 2, 1935. The use of aluminum has proved of distinct benefit to Valencia orange cuttings when the phosphate treatment is the same in all cultures. Growth was poorest when neither zinc nor aluminum was added; growth was best for the period investigated when aluminum was added without zinc. The use of zinc without aluminum gave better growth than when both zinc and aluminum were omitted.

Many experiments have indicated the beneficial effect of aluminum on the growth of citrus in solution cultures. Because of this fact, lemon leafy-twig cuttings were grown in the best grade "Wearever" aluminum kettles of approximately 15-liter capacity. The overlapping lids were also made of aluminum and, by means of clamps, were tightly joined with the kettles, thus effectively keeping dust out of the culture solutions. Although aluminum is known to remove copper completely from solutions, the age of these cuttings has not brought this element into a deficiency consideration as yet.

The aerated cultures were started on February 6 and were photographed on September 2, 1935. During the experiment the solution used for culture *A* (pl. 2, fig. 3) contained 105 p.p.m. phosphate continually, while that used for culture *B* (pl. 2, fig. 3) contained 105 p.p.m. phosphate for only a few days at intervals of several weeks. It was assumed that during the absence of phosphate in the culture solution of *B* there may have been sufficient aluminum and impurities in the aluminum made soluble to give an impetus to the growth of the cuttings. The roots in both cultures were healthy in appearance, those in *A* being noticeably shorter than those in *B*. In *A* the tops were much branched and the leaves were large and healthy in appearance. In *B* the shoots were mostly unbranched; many of the leaves were of small size, and a few showed a burn that is characteristic of a phosphorus deficiency. Cultures such as *B* that receive phosphate at intervals do not ordinarily show burn when grown under the same conditions in enameled pans or in glass containers instead of aluminum. This fact and the phosphorus content of leaves, shoots, and roots (numbers 5 to 8 in table 2) indicate that with aluminum containers

a shorter interval may be necessary between the periods of phosphate absorption.

SUMMARY

By means of solution cultures, a study was made of the phosphorus nutrition of citrus and its relation to aluminum. The use of calcium aluminate was beneficial not only to the roots but also to the tops of lemon seedlings.

The beneficial effect of phosphate on lemon leafy-twig cuttings was largely in the growth of the tops when the culture solution was aerated. When aluminum was present the roots were healthy and more extensive, but the tops usually were retarded in growth.

Excellent growth of citrus in solution cultures was obtained by the use of 105 p.p.m. of phosphate supplied for a few days at intervals of several weeks. Very little growth may occur in nonaerated cultures when phosphorus is deficient, although the roots remain in a healthy state. Aluminum greatly improved the root systems of Lisbon leafy-twig cuttings grown without phosphate. When phosphate was present for a few days at intervals of several weeks, aluminum benefited the root system at the apparent expense of the top growth.

Root caps were numerous when citrus roots were grown in the presence of aluminum in the culture solution.

Recovery experiments showed that lateral buds inhibited by a phosphate deficiency resumed growth when phosphate was supplied.

Extreme symptoms of phosphorus deficiency were accompanied by low percentages of phosphorus in the plant tissues.

The percentage of phosphorus in the original leaves of citrus cuttings was subject to considerable change, depending on the phosphate content of the culture solution.

Aluminum was beneficial to the early growth of Valencia orange leafy-twig cuttings whether or not zinc was added to the culture solution.

When aluminum containers were used as culture vessels, the roots were healthy in appearance and were much shorter when 105 p.p.m. phosphate was continually supplied than when the phosphate was present for only a few days at intervals of several weeks.

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PLATE 1

Effect of Aluminum and Phosphate on Growth of Lemon Plants

Fig. 1. Aluminate as a better source of calcium than citrate for the growth of lemon seedlings in 2-quart Mason jars filled with nonaerated culture solution containing phosphate. Three corks to the left, seedlings grown in a culture solution lacking calcium; three corks in the center, seedlings grown in a similar solution plus 1 gm. of calcium citrate to each jar; three corks to the right, seedlings grown in a solution similar to that used for the seedlings on the extreme left plus 1 gm. of calcium aluminate (Kahlbaum) to each jar.

Fig. 2. Lisbon lemon leafy-twig cuttings grown in nonaerated culture solutions without aluminum: *A*, phosphate-free culture solution; *B*, 105 p p m. phosphate present for only a few days at intervals of several weeks.

Fig. 3. Lisbon lemon leafy-twig cuttings grown in nonaerated culture solutions with aluminum: *A*, phosphate-free culture solution to which 10 p.p.m. aluminum was added as the nitrate; *B*, 105 p.p.m. phosphate present for only a few days at intervals of several weeks and 10 p.p.m. aluminum added as the nitrate when phosphate was absent. Compare figures 2*A* and 3*A*; also 2*B* and 3*B*.

Fig. 4. Eureka lemon leafy-twig cuttings grown in nonaerated cultures from April 11 to December 26, 1933, in enameled pails of 9-liter capacity. The pH was maintained at 4.5 with dilute nitric acid. *A*, 105 p.p.m. phosphate present in the culture solution for a few days at intervals of several weeks, right, 10 p.p.m. aluminum added as the citrate when phosphate was absent; left, same, except 20 p p.m. aluminum. *B*, aluminum absent· left, phosphate absent, right, 105 p.p.m. phosphate always present.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

PLATE 2

EFFECT OF ALUMINUM AND PHOSPHATE ON GROWTH OF LEMON AND ORANGE PLANTS

FIG. 1. Root caps on the roots of lemon cuttings grown in a culture solution containing no phosphate and to which 5 to 10 p.p.m. of aluminum was added as the nitrate.

FIG. 2. Beneficial effect of aluminum on the growth of Valencia orange leafy-twig cuttings in culture solutions containing 105 p p m phosphate for a few days at intervals of several weeks. When phosphate was omitted the culture solution contained, left to right, no zinc, no aluminum; 1 p.p m. zinc, no aluminum, 1 p p m. zinc, 1 p p.m aluminum; no zinc, 1 p p m aluminum

FIG. 3. Effect of continuous and intermittent phosphate in the culture solution on the growth of lemon leafy-twig cuttings in aluminum containers A, 105 p.p.m. phosphate continually, B, 105 p p.m. phosphate for a few days at intervals of several weeks

Fig. 1

Fig. 2

Fig. 3

UNPRODUCTIVENESS OF CERTAIN ORCHARD SOILS AS RELATED TO LEAD ARSENATE SPRAY ACCUMULATIONS[1]

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A rapidly increasing number of experiences with unsatisfactory crop growth for one to several years following the removal of old apple trees in certain orchards in central Washington have been reported in recent years. Some of these experiences are summarized by Snyder (7). The difficulty seems to occur most generally on orchard land from which trees intensively treated with insect sprays for a period of years have been removed. Headden (4) ascribed the death of many apple and pear trees in Colorado to arsenical poisoning resulting from heavy applications of arsenical sprays. Since the prevailing spray practice in the orchards of central Washington may result in considerable accumulations of lead arsenate compounds in the soil, an investigation of the relation of the concentration of these compounds in the soil to its productivity was begun in May, 1935. The preliminary results are reported in this paper.

EXPERIMENTAL PROCEDURE

A field in the Yakima Valley from which 27-year-old apple trees were removed in 1932 was selected for study. This field lay idle until the spring of 1934, when it was seeded to alfalfa with barley as a nurse crop. The alfalfa germinated poorly, and the barley plants emerged but soon turned yellow and the majority died when they reached a height of about two inches. The field was plowed in the fall and reseeded to alfalfa and barley the following spring. Again very little alfalfa emerged, and the stand of barley was exceedingly spotted, some of the spots supporting apparently normal plants. On May 14, representative samples of the surface 6-inch layer and also of the underlying 6 to 12-inch layer of soil in several apparently normal as well as poor spots were obtained for analysis. Measured quantities of one of the samples derived from the poor spots were extracted with various solutions for the purpose of finding a suitable means of determining readily soluble arsenic. One

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part of soil was mixed with five parts of cold distilled water or with a 0.1 *N* solution of various salts and allowed to stand for 4 hours with occasional shaking. The suspensions were filtered, and aliquots of the filtrates were analyzed for arsenic by the Gutzeit (1) method. The results are reported in table 1. As can be noted, the amounts of readily soluble arsenic extracted by the various solutions correlated closely with one another with the exception of those extracted by the ammonium carbonate and potassium solutions. Since flocculation of the soil is desirable to speed up filtration, the 0.1 *N* am-

TABLE 1

Influence of extracting solutions on the amount of readily soluble arsenic

EXTRACTING SOLUTION	As_2O_3
	p.p.m.
H_2O	3.5
0.1 *N* NH_4Ac	3.7
0.1 *N* NH_4NO_3	3.6
0.1 *N* $(NH_4)_2SO_4$	3.9
0.1 *N* $(NH_4)_2CO_3$	9.6
0.1 *N* KNO_3	4.1

TABLE 2

Relation of readily soluble arsenic to that soluble in hot concentrated HNO_3

FIELD	SAMPLE	DEPTH	CROP CONDITION	As_2O_3	
				Soluble in concentrated HNO_3	Soluble in cold 0.1 *N* NH_4Ac
		inches		*p.p.m.*	*p.p.m.*
1	1	0–6	poor	48.0	3.0
		6–12		17.5	0.8
	2	0–6	poor	48.6	9.8
		6–12		7.5	3.0
2	3	0–6	normal	13.3	0.8
		6–12		3.2	0.3
	4	0–6	normal	4.0	0.3
		6–12		1.6	0.1

monium acetate solution was selected for all subsequent determinations of readily soluble arsenic, one part of soil being used to five parts of solution.

RELATION OF READILY SOLUBLE ARSENIC TO ARSENIC SOLUBLE IN CONCENTRATED HNO_3

Suitable quantities of soil from two samples derived from normal spots and two samples derived from poor spots were extracted with cold 0.1 *N* ammonium

acetate and concentrated hot nitric acid, respectively, those extracted with nitric acid being digested on a steam bath for 3 hours, one part of soil being used to five parts of acid. The filtrates of the hot nitric acid extracts were diluted sufficiently to overcome interference of the nitrate ion with the arsenic determination, and aliquots of the diluted filtrates as well as of the ammonium acetate extracts were analyzed for arsenic. The results are presented in table 2.

If it is assumed that a large part of the arsenic residue in the soil was recovered by digestion in concentrated HNO_3, the data indicate the presence of considerable quantities of this material and the accumulation of the bulk of it in the surface layer. The greatest concentration of readily soluble arsenic also seemed to occur in the upper 6 inches. Moreover, the condition of the crop as observed in the field was closely related to the amount of readily soluble arsenic in the surface layer of soil.

TABLE 3

Relation of depth to readily soluble arsenic

DEPTH	As_2O_3	
	Field 1	Field 2
inches	*p.p.m.*	*p.p.m.*
0–6	3 7	7 0
6–12	1 2	2 5
12–24	1 0	0 6
24–36	0 7	1 3
36–48	0 8	0 4

AMOUNTS OF READILY SOLUBLE ARSENIC AT VARIOUS DEPTHS IN THE SOIL

Samples of soil taken at various depths from the surface down to 48 inches were obtained from two fields, field 1 being the same from which the previous samples were obtained, and field 2, one located a few miles distant where the removal of old apple trees was followed by poor growth of the subsequent crop. The amounts of readily soluble arsenic in these samples were determined, and the results are given in table 3. The data show that in both soils the greatest concentration of readily soluble arsenic occurred in the surface 6-inch layer and decreased greatly with depth.

RELATION OF READILY SOLUBLE ARSENIC AND TOTAL WATER-SOLUBLE SALT CONTENT TO CROP BEHAVIOR

Samples of soil taken from the surface 6 inches and from a depth of 6 to 12 inches were obtained from a number of fields in the Yakima Valley where old fruit trees had been removed and where subsequent crop growth indicated various degrees of unproductiveness. One field which has never been in

orchard was included as a control. The texture of the soils ranged from fine sandy loam to silt loam, and all the fields selected were free from abnormal

TABLE 4

Relation of readily soluble arsenic and total water-soluble salt content to crop behavior

FIELD NUMBER	CROP HISTORY	CROP	CONDITION OF CROP	DEPTH	TOTAL WATER-SOLUBLE SALTS	READILY SOLUBLE As_2O_3
				inches	*p.p.m.*	*p.p.m.*
1	Field in alfalfa for many years, never planted to orchard	Barley and alfalfa	Very good	0–6 6–12	205 188	trace trace
2	Apple trees until 15 years ago. No lead arsenate spray since. Peach and prune trees until removed a few years ago. Seeded in 1935	Barley and alfalfa	Good	0–6	284	0 6
3	Old apple trees removed in 1932, 6 in. of surface removed in leveling	Alfalfa	Good	0–6	176	0.1
4	Jonathan apple trees removed in 1932. Amount of lead arsenate spray about one-half usual amount. Alfalfa successful in 1932. Reseeded in 1935	Alfalfa	Good	0–6 6–12	157 195	1 1 1 0
5	Old apple trees removed in 1933. Oats and alfalfa failure in 1934. Reseeded in 1935	Oats and alfalfa	Very poor	0–6 6–12	351 314	8 2 5 2
6	Apple trees removed in 1934 after the orchard was neglected and little lead arsenate spray used for 5 years. Strip plowed in 1934 and seeded in 1935	Barley and alfalfa	Fair	0–6 6–12	237 247	1 5 1 9
	Strip plowed in 1935, seeded in 1935	Barley and alfalfa	Very poor	0–6 6–12	527 752	16 3 10 4
	Strip along head ditch filled after trees trees were removed. Seeded in 1935	Barley and alfalfa	Good	0–6 6–12	271 258	1 1 0 1
7	Jonathan apple trees 32 years old removed in 1933, sloping area. Seeding of alfalfa in 1934 failed. Reseeded in 1935	Alfalfa	Poor	0–6 6–12	176 166	4 5 3 0
	Area at bottom of slope. Seeded in 1935	Alfalfa	Very poor	0–6 6–12	166 202	12.5 4 2
8	Old Bartlett pear trees removed in 1933. Alfalfa seeded in 1934, fair results. Reseeded in 1935	Alfalfa	Good	0–6 6–12	237 174	1 9 0 4
9	Old apple trees removed in 1933. Seeding of barley in 1934 failed. Reseeding in 1935 failed. Clover was then tried	Clover	Fair	0–6 6–12	202 189	2.6 1 1
	Releveled area part of surface soil removed. Seeded in 1935	Clover	Fair	0–6 6–12		3 2 2.2

alkali salt accumulations. As a further check in this regard the total soluble salt content of the samples was determined by means of the Wheatstone bridge (2). As previously, the readily soluble arsenic content of the 0.1 N NH_4Ac

extracts was determined. The results are recorded in table 4 together with a brief sketch of the previous cropping history, a notation of the crop growing at the time the soil samples were obtained, and the condition of this crop.

The data reveal that the concentration of water-soluble salts was not abnormally high in any of the soils and that there was a close relationship between the behavior of the crop and the readily soluble arsenic content in the surface foot of soil.

When the concentration of readily soluble arsenic in the upper 6-inch layer exceeded 2 p.p.m., marked damage to normal growth of crops resulted. Barley and alfalfa seemed to suffer severe injury or even death when the concentration of readily soluble arsenic in the surface 6 inches of soil exceeded 3 p.p.m. It is noteworthy also that in general the greatest concentration of readily soluble arsenic occurred in the surface 6-inch layer, which is the plowed layer and the layer in which the root system of young plants develops. In many of the fields where young plants suffered it was not unusual to find normal, luxuriant, old alfalfa plants which were there before the trees were removed and had escaped the action of the plow. The fact that the root system of the old plants was established beneath the layer in which the spray residue was concentrated may explain their normal appearance.

EFFECT OF DIFFERENT SOILS AND SOIL TREATMENTS ON THE BEHAVIOR OF BARLEY

Suitable amounts of soil were obtained from the surface 6 inches of representative areas in each of three fields in the Yakima Valley. The field represented by soil 1, which never was in orchard, is productive. Three attempts to grow barley or alfalfa on the field represented by soil 2 failed following the removal of apple trees in 1932. Likewise the two attempts to obtain a stand of alfalfa with barley as a nurse crop in the field represented by soil 3 failed following the removal of the apple trees in the spring of 1934.

The bulk samples of these soils were thoroughly mixed for use in pot culture experiments in the greenhouse with the view to determining the effect of additions of various salts on the toxic effect of the soils and to ascertaining more specifically the cause of the toxicity. Analyses were made for soil reaction, for total water-soluble salts, for arsenic soluble in both hot concentrated HNO_3 and cold 0.1 *N* NH_4Ac, and for lead soluble in both hot concentrated HNO_3 and cold 0.1 *N* NH_4NO_3. The procedure for preparing the soil extracts for lead determinations was identical with that for arsenic. After proper checking with the sodium sulfite method, the diphenylthiocarbazone method (8), because of its adaptability for determinations of small quantities, was employed for lead analysis of suitable aliquots of the filtrates of the soil extracts. Ammonium acetate was replaced by ammonium nitrate as an extracting agent because of the desirability of having the lead compounds in the nitrate form for this method. The results of the various analyses are given in table 5.

As may be noted from the data in this table, the total soluble salt content

was not abnormally high in any of the soils, but the reaction of soil 3, which had a pH value of 8.10, suggests the presence of some black alkali salts. This apparently enhanced the solubility of both arsenic and lead, particularly the former. The contents of arsenic and lead soluble in hot concentrated HNO_3

TABLE 5

Reactions, soluble salt content, and contents of lead and arsenic of soils obtained from three different fields

SOIL NUMBER	pH VALUES*	WATER-SOLUBLE SALTS	As_2O_3 SOLUBLE IN HOT HNO_3	As_2O_3 SOLUBLE IN COLD 0.1 N NH_4Ac	PbO SOLUBLE IN HOT HNO_3	PbO SOLUBLE IN COLD 0.1 N NH_4NO_3
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	6.75	108	trace	trace	4 95	0 07
2	7.40	202	44 0	3.7	64 6	0 24
3	8.10	315	41 0	7.0	49 6	0 24

* Determined by means of the glass electrode.

TABLE 6

*Effect of different soils and soil treatments on the yield and behavior of barley**

SOIL NUMBER	DETERMINATION	CONTROL	10 M.E. $CaCO_3$	50 M.E. $CaCO_3$	10 M.E. $CaSO_4$	50 M.E. $CaSO_4$	10 M.E. $FeSO_4$	50 M.E. $FeSO_4$
1	Dry wt. of tops.......*gm.*	4 67	4 40	6 35	4 40	4 17	4 41	5.84
	PbO in tops .... .*p.p.m.*	none	none	none	none	none	none	none
	As_2O_3 in tops . .. .*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	Dry wt. of roots... ...*gm.*	0.86	1.06	2.67	1.25	2 33	1 52	1.70
	PbO in roots.... ..*p.p.m.*	none	trace	trace	trace	trace	none	trace
	As_2O_3 in roots .....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
2	Dry wt. of tops.......*gm.*	1 00	1 73	2.05	2 49	3 19	3 40	3 97
	PbO in tops... ....*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	As_2O_3 in tops.......*p.p.m.*	10 01	2 54	5.78	4 01	2 51	2 52	1 48
	Dry wt. of roots ... ..*gm.*	0 53	0.65	0 97	1.13	1 31	1 18	1 79
	PbO in roots.......*p.p.m.*	662 00	392.00	354 00	315 00	214 00	352 00	208 00
	As_2O_3 in roots......*p.p.m.*	788.00	472 00	112 50	91 50	87.50	89 60	30 50
3	Dry wt. of tops ... . .*gm.*	0 57	1 03	0 54	0.76	0 60	2 02	4 28
	PbO in tops... . ..*p.p.m.*	trace	trace	trace	trace	trace	trace	trace
	As_2O_3 in tops.......*p.p.m.*	17 50	7.76	16 75	9.75	9 92	9.34	6.55
	Dry wt. of roots.......*gm.*	0.21	0.42	0.10	0.34	0 37	1.07	1.67
	PbO in roots.......*p.p.m.*	756.00	554 00	551 00	387 00	354 00	275.00	112.50
	As_2O_3 in roots......*p.p.m.*	1,640.00	287.00	1,540 00	239.00	198 00	102.00	72.00

* The yields of tops and roots are those from one pot containing six plants.

were less in soil 3 than in soil 2; whereas the readily soluble arsenic content was greater in the former than in the latter and both contained equal amounts of readily soluble lead. Headden (4) observed similar effects of alkali salts on the solubility of arsenic in Colorado soils. Soil 1, which is productive and never received any lead arsenate spray, was practically free from arsenic and

contained only a very small amount of lead. These facts should be significant in connection with the toxic effects on crops produced on these soils.

Duplicate 2-kgm. portions of soil from the bulk samples of these three fields were mixed with various salts selected in an attempt to reduce the solubility of the accumulated arsenic compounds in the soil. The salts, which were applied on the basis of milliequivalents per 100 gm. of dry soil, were incorporated by thorough mixing. The soils were then placed in pots and seeded to barley. The plants in each pot were thinned to six a few days after they emerged. When the crop reached the blooming stage of maturity, the tops and roots were harvested separately, dried, and weighed. Samples of oven-dried top and root material were digested with concentrated 1:1 HCl and $KClO_3$ according to the method given by Scott (5), and determinations were made for arsenic and lead on aliquots of the filtrates of the digests. The analytical results together with the treatments and yields of dry matter from one pot in each treatment are recorded in table 6.

The condition of the barley plants produced on soil 1 was normal in every respect and, as noted from the data in table 6, was not greatly affected by the various salt treatments. The plants on the control pots containing the other two soils developed leaves with yellow tips soon after emergence, some of the affected leaves drying up completely later. These plants never passed through the shooting and heading stages and barely managed to survive. The toxic symptoms of the plants produced in the greenhouse were identical with those observed on plants in the affected fields. These symptoms together with the extremely low yield of dry matter produced are ample evidence of a distinctly toxic condition in these soils. The severity of the toxic effects in soil 2 was notably ameliorated as a result of treatments with $CaCO_3$, $CaSO_4$, and $FeSO_4$, especially the last. In soil 3, in which the toxic effect appeared to be greater than in soil 2, the treatments with $CaCO_3$ and $CaSO_4$ produced little or no beneficial effect. Both treatments with $FeSO_4$, however, proved to be distinctly beneficial. It is possible that the beneficial effects on soils 2 and 3 resulting from the treatments with calcium and iron salts, especially the latter, were caused by conversion of some of the readily soluble arsenic to less soluble forms.

When the data in table 5 are considered, it is noteworthy that in general the yield of tops on the control soils was inversely proportional to the amounts of readily soluble arsenic in the soil and the arsenic contents of the aerial parts of the plants. It is noted also that the arsenic content of the roots of the affected plants was very high.

Although the amounts of readily soluble lead in the affected soils were very small, nevertheless the roots of the barley plants produced on these soils were capable of absorbing large quantities. Evidently only traces of the absorbed lead were translocated to the aerial parts. A similar localization of lead in plants was observed by Hammet (3), who concluded that increasing concentrations of soluble lead in the nutrient medium result in proportional retarda-

tion of the root growth. Since the concentration of readily soluble lead in the soils indicated in table 6 was low, it would seem that the toxicity produced by the accumulation of lead arsenate in the soil was caused chiefly by excessive concentrations of readily soluble arsenic. An attempt to clarify this point further was made by means of the following experiment.

EFFECT OF DIFFERENT CONCENTRATIONS OF ARSENIOUS ACID, LEAD NITRATE, AND LEAD ARSENATE ON THE BEHAVIOR OF BARLEY

A number of 2-kgm. samples of dry soil were taken from the supply of soil 1 used in the preceding experiment. One series of samples was thoroughly mixed with various amounts of arsenious acid together with a sufficient quantity of ammonium nitrate to make the nitrogen content equivalent to that contained in the lead nitrate required to supply the equivalent of 50 p.p.m. PbO. Another series of samples was thoroughly mixed with various amounts of lead nitrate. In addition to these treatments duplicate samples received 50 p.p.m. lead arsenate and the same quantity of ammonium nitrate as was added to the arsenious acid treatments. Two additional samples were used as controls but to make them comparable to the others with respect to nitrogen they were given the same amount of ammonium nitrate as the arsenious acid treated samples. The soils were placed in pots, and enough water was added to adjust the moisture content to approximately the normal moisture capacity described by Shaw (6). The pots were kept for 3 days in this condition to allow time for fixation and adjustment of the added compounds. Soil samples were then obtained for readily soluble arsenic and lead determinations, and the pots, after being planted to barley, were moved to the greenhouse. Two months later when the plants were well in the shooting stage of development samples consisting of a core of soil taken from the center of each pot were obtained for analysis. The soil treatments together with the analytical results and a brief description of the condition of the plants are recorded in table 7. The condition of the plants at the age of 10 weeks is illustrated in plate 1.

Although highly soluble arsenic and lead compounds were used in this experiment, it was found that on account of fixation by the soil only very small amounts could be recovered in readily soluble form at the end of 3 days. Determinations of readily soluble arsenic and lead made on a few samples 2 hours after they were treated yielded similar amounts, indicating that the fixation process took place rapidly. From the results obtained from the analyses of the soil samples taken at the shooting stage of the barley, it appears that the concentration of readily soluble arsenic and lead did not change greatly during the course of the experiment. In general the concentration of readily soluble arsenic had a tendency to increase slightly, but that of lead, which was already very low at planting time, suffered a further reduction.

The behavior of the plants demonstrated clearly that readily soluble arsenic in certain concentrations is toxic to barley. The toxic symptoms were identi-

cal with those manifested by the barley in the affected fields and in the preceding plant culture experiment. Moreover, the degree of toxicity exhibited

TABLE 7

Effect of soil treatments with different amounts of arsenious acid, lead nitrate, and lead arsenate on the behavior of barley

NUMBER	TREATMENT	READILY SOLUBLE ARSENIC IN P.P.M. OF As_2O_3		READILY SOLUBLE LEAD IN P.P.M. PbO		CONDITION OF PLANTS
		Planting time	Stooling stage	Planting time	Stooling stage	
1 & 2	NH_4NO_3*	trace	trace	0 07	trace	Normal, vigorous plants
3 & 4	50 p.p.m. lead arsenate	0 04	0 10	0 06	0.03	Normal seedlings, very slight symptoms of toxicity at shooting stage
	Arsenious acid					
5	50 p.p.m. As_2O_3*	trace	0 20	...	....	Normal seedlings, very slight symptoms of toxicity at shooting stage
6	60 p.p.m. As_2O_3*	0 10	0 50	...	.. .	Normal seedlings, slight yellowing of leaf tips later, slight symptoms of toxicity at shooting stage
7	120 p p m. As_2O_3*	1 00	2 00			Normal seedlings, some yellowing of leaf tips later with distinct signs of toxicity, distinct toxicity at shooting stage
8	180 p.p m. As_2O_3*	5 00	7 00	....	....	Seedlings with yellow tips, stunted growth, leaves drying, plants failed to pass through shooting stage
9	240 p p.m. As_2O_3*	25 00	20 00			Seedlings with yellow tips, no appreciable growth, plant barely survived
	Lead nitrate					
10	50 p.p.m. PbO			0 05	0 02	Normal, vigorous plants
11	60 p.p.m. PbO			0 10	0 05	Normal, vigorous plants
12	120 p.p.m. PbO		..	0 16	0 05	Normal, vigorous plants
13	180 p.p.m. PbO			0 21	0 07	Normal, vigorous plants
14	240 p.p.m. PbO	..	. .	0 37	0 14	Normal plants, vigorous growth but slight tendency to yellowing of lower leaves during shooting stage

* Sufficient ammonium nitrate was added to make the nitrogen content equivalent to the amount of lead nitrate supplying the equivalent of 50 p.p.m. PbO.

by the plants in this experiment was proportional to the concentration of readily soluble arsenic in a similar manner to that observed in the affected

fields. No marked toxic effects were evident when the concentration of readily soluble arsenic in the soil was less than 1 p.p.m., but the toxic action was severe where the concentration was 5 p.p.m. or more.

It is noteworthy that the amounts of soluble lead in the lead nitrate treated soils were exceedingly small. No distinct symptoms of toxic effects were produced by this compound although the growth of the barley was retarded, especially on the soils receiving 60 and 120 p.p.m. of PbO as lead nitrate respectively. Possibly concentrations exceeding 240 p.p.m. PbO might have shown toxic symptoms.

When the results of these experiments are considered as a whole, it appears that one of the chief causes of the unproductiveness of the orchard soils investigated is the excessive concentration of readily soluble arsenic which resulted from the arsenate spray accumulations. It is possible also that readily soluble lead, if liberated in sufficient concentrations from the accumulated spray compound in the soil, may be a contributing factor. Probably the exact concentration of each of these substances that will produce toxic effects on crops varies considerably, depending upon the kind of crop grown and the inherent characteristics of different soil types. Investigations of the soluble arsenic and lead tolerances of various crops on different soil types and of methods of overcoming the toxic effects of these substances are in progress and will be reported later.

SUMMARY

The cause of the unproductiveness of certain soils in the Yakima Valley from which old apple trees have been removed in recent years was investigated.

Soil samples obtained from the surface 6 inches of a number of unproductive fields contained from 4.5 to 12.5 p.p.m. of readily soluble arsenic calculated as As_2O_3. The poor condition of young alfalfa and barley in the affected fields seemed to be roughly in proportion to the concentration of readily soluble arsenic in the surface soil.

The symptoms of toxicity of barley grown in the greenhouse in pots filled with untreated soil taken from the surface 6 inches in two unproductive fields were identical with those observed in the field. The arsenic content of samples of tops and roots of this barley harvested at the blooming stage of maturity ranged from 10.01 to 17.50 p.p.m. of As_2O_3 in the tops, and from 788 to 1640 p.p.m. in the roots. Only traces of PbO were found in the tops; whereas in the roots the amounts ranged from 662.50 to 756 p.p.m.

Samples of soil free from accumulations of readily soluble arsenic and lead, and treated with various amounts of arsenious acid and lead nitrate, respectively, were used for greenhouse culture work. The toxic symptoms of the barley plants growing in samples containing readily soluble arsenic equivalent to 5 p.p.m. or more of As_2O_3 at planting time were identical with those observed on barley in the affected fields. Retardation of growth occurred, though no definite toxic effects on barley seemed to result in soil samples receiving more than 50 p.p.m. of lead nitrate.

The results as a whole indicated that one of the chief causes of the unproductiveness of the orchard soils investigated appears to be the excessive concentration of soluble arsenic resulting from lead arsenate spray accumulations in the surface soil. Soluble lead, if liberated in sufficient concentration from the accumulated spray compounds in the soil, may be a contributing factor.

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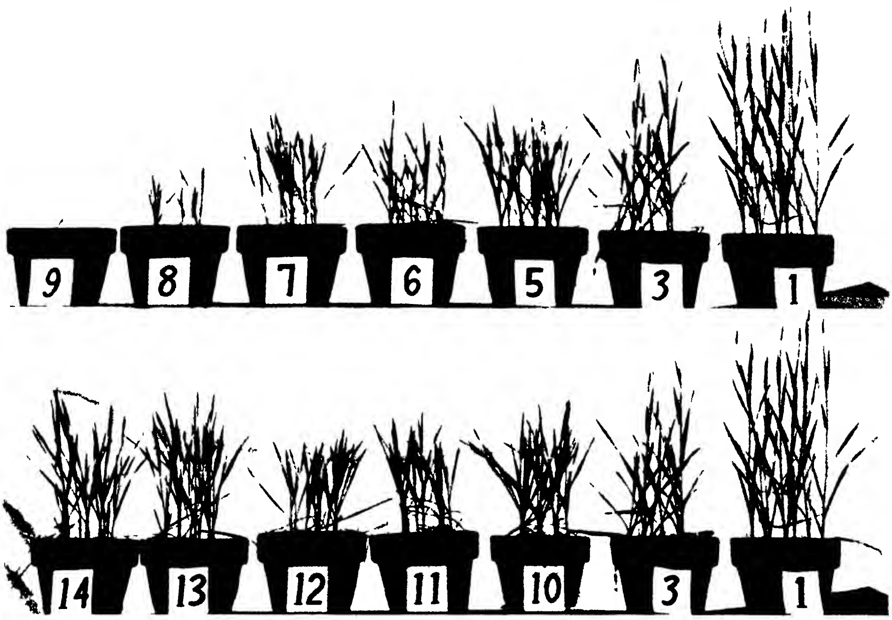
PLATE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF LEAD ARSENATE, ARSENIOUS ACID, AND LEAD NITRATE ON BARLEY PLANTS

1, check; 3, 50 p.p.m. lead arsenate; 5, 50 p.p.m. As_2O_3; 6, 60 p.p.m. As_2O_3; 7, 120 p.p.m. As_2O_3; 8, 180 p.p.m. As_2O_3; 9, 240 p.p.m. As_2O_3; 10, 50 p.p.m. PbO, 11, 60 p.p.m. PbO; 12, 120 p.p.m. PbO; 13, 180 p.p.m. PbO; 14, 240 p.p.m. PbO.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THE DILATOMETER METHOD AS AN INDIRECT MEANS OF DETERMINING THE PERMANENT WILTING POINT OF SOILS[1]

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Of all the moisture constants that are usually determined, the wilting point has the most meaning and practical importance. Probably the main reason it has not been employed heretofore as much as it should in the examination of soils and in moisture studies is that the present direct method is laborious and time-consuming. There is, therefore, a great need for a practical, rapid, and accurate method for determining the wilting point of soils.

The dilatometer method gives promise of being a satisfactory indirect means for determining the wilting point of soils (1). It is the purpose of this paper, therefore, to describe this method, its principle and technique, and to show how the results obtained by it compare with those obtained by the direct method.

PRINCIPLE OF THE METHOD

The principle of the dilatometer method in determining the wilting point of soils is based upon the fact that when wet soils are super-cooled to −1.0°C. and are caused to freeze, a certain amount of the water contained in the soil fails to freeze, the amount depending upon the kind of soil. The amount of water failing to freeze at −1.0°C. is the same as that at which plants permanently wilt as determined by the direct method. The amount of water freezing is measured on the principle of expansion. According to the dilatometer used, 1 cc. of water at 0°C. expands 0.1 cc. upon freezing. When the total amount of water present is known, the amount of water that freezes or fails to freeze can thus be readily calculated.

PROCEDURE OF THE METHOD

The following materials are required for making a wilting point determination by the dilatometer method:

Dilatometer (pl. 1) having a 60 cc. capacity bulb and a 1.1 cc. stem graduated into 0.01 cc.
Small cork stoppers.
Ligroin of high boiling point.
Thermometer which has accurate reading between 0 and −10°C.

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A standard arrangement on the water faucet to produce suction force by the running water.
A 10-cc. and a 5-cc. pipette.
An accurate balance.
Ice and salt.
Two baths, made by putting a small crock inside a larger one, insulating the space between them with rock wool and making connections water tight.
Tallow.
Alcohol for drying the dilatometer with the aid of the suction force.

The procedure for making a wilting point determination by means of the dilatometer method consists of placing into the dilatometer by means of a small funnel 20 gm. of air-dry soil which has been passed through a 2-mm. sieve. To sand, 5 cc. of distilled water is added by means of an accurate 5-cc. pipette. To loam or clay loam, 10 cc. of water is added. If the soil is a very heavy clay or one that contains a very high content of organic matter, 5 cc. of water is first added to the dilatometer before the soil is poured in, and then 5 cc. is added on top of the soil after it is poured in. This procedure usually insures a complete wetting of the entire soil mass without stirring. After the soil is allowed to stand for about 20 minutes to absorb the water, the dilatometer is filled half full with ligroin, stoppered, and the stem is connected to the suction force at the water faucet for a few seconds to remove the air from the soil. Care must be taken not to turn on a too strong suction force which will suck away the ligroin and water. Likewise, in order to prevent the water from backing up, the suction force must be disconnected at the stem of the dilatometer and not by turning off the faucet. Sometimes it may be necessary to stir the soil. In this case the stirring is done with a thin sturdy wire, and then that portion of the wire which touches the soil is cut off by a pair of plyers and left in the dilatometer. When this procedure of suctioning and stirring the soil is followed, no loss of water takes place.

After the soil is freed of air, the dilatometer is filled with ligroin and stoppered with the cork stopper, the outside of which has been smeared with tallow to prevent any absorption of water. Whenever necessary, additional ligroin is added to the dilatometer through the stem. By holding the stem at a slight angle and adding the ligroin in small drops, the formation of bubbles in the stem is easily avoided. An attempt should always be made to maintain the level of the ligroin column near the zero point so that when the water freezes and expands, the column will not be beyond the scale.

The dilatometer is then placed in the cold bath, having a temperature of exactly -1.0°C., and left until it attains the temperature of the bath, as indicated by the ligroin column's becoming constant. As the temperature of the bath does not remain uniform throughout without frequent stirring, an effort is made to stir the mixture almost continuously. An excellent plan is to put into the bath about 6 dilatometers, or as many as it will hold, and then, by holding the stems together at the top, move them up and down in the cold mixture. This procedure not only serves to stir the mixture and keep its temperature uniform, but also helps the dilatometers to attain the temperature

of the bath faster. When the dilatometers are moved in the cold mixture, some soils may solidify prematurely. In such a case the dilatometer is taken out and warmed and the position of the soil is moved slightly by gentle tapping.

When the ligroin column in the stem has become constant after several readings, the dilatometers are placed in a separate bath having a temperature of about -10.0°C. The dilatometers are left in this bath for about 25 minutes in order that all the free water in the soil may freeze, as at the temperature of -1.0°C. it freezes very slowly and incompletely. The dilatometers are then transferred to the original bath of -1.0°C. and left in this temperature with almost continuous stirring until they attain the temperature of the bath and the ligroin column becomes constant. The excess water frozen at -10°C. over that frozen at -1.0°C. remelts quickly at the latter temperature, as indicated by the descent of the column after it reaches a certain peak, and only that portion of the water on which the soil exerts an influence remains frozen at -1.0°C. In this manner the amount of water that remains frozen at -1.0°C. becomes absolute and accurate.

The final reading, after freezing, is recorded, and from this reading is subtracted the first reading before freezing; the difference is the number of cubic centimeters of the added water that froze. By subtracting this latter amount from the amount of water that was added to the soil, one obtains the quantity of water that failed to freeze. To the latter is added also the hygroscopic water. This total amount of water is divided by the amount of soil used on the oven-dry basis, which gives the total percentage of water that failed to freeze at -1.0°C., which is considered to be the permanent wilting point of the soil.

This method is simple and very rapid. After the soil is prepared in the dilatometer it takes only about one hour to obtain the final dilatometer reading from which the wilting point is calculated. Since the dilatometer is small in volume, as many as eight dilatometers can be placed in a bath 7 inches in diameter; consequently, determinations on as many as eight soils can be run at the same time. It is possible to determine the wilting point of as many as sixteen soils in a single day.

The method is also accurate, as it gives reproducible results to within 1 per cent. The following potential sources of error, however, should be guarded against:

Premature solidification: This is easily recognized and easily remedied. It occurs in only a few soils.

Failure of all the free water to freeze at -10°C.: If the soils are left at -10°C. for about 25 minutes, all the free water will freeze.

Failure to maintain a uniform temperature in the bath: With the plan suggested, the temperature easily can be maintained uniform.

Failure to expel all the air bubbles in the soil: Probably this is the greatest source of error, especially in some particular soils which have a tendency to produce bubbles when they come in contact with the water and the ligroin. With care, however, the suction method suggested can expel all the air bubbles.

EXPERIMENTAL

In order to ascertain whether the dilatometer method as described can actually determine the wilting point of soils, a comparison was made of this method

TABLE 1

A comparison of the permanent wilting point as obtained by the direct method and by the dilatometer method

SOIL DESIGNATION	NAME OF SOIL	ADDED WATER FAILED TO FREEZE	HYGROSCOPIC WATER	TOTAL WATER FAILED TO FREEZE OR PERMANENT WILTING POINT BY DILATOMETER METHOD	PERMANENT WILTING POINT BY DIRECT METHOD*
		per cent	*per cent*	*per cent*	*per cent*
CL	Columbia silt loam	6 35	2 27	8 62	8.61
OS	Delano silt loam	3 75	1 35	5.10	4 17
FAL	Farwell silt loam	10 55	3 84	14 39	14 14
FS	Fresno sandy loam	2 60	0 67	3 27	3 05
G	Columbia silt loam	5 65	1 32	6 97	6.69
H	Stockton clay adobe	7.00	2.31	9 31	9 28
HS	Harford fine sandy	4.50	0.77	5 27	3.99
L	Columbia sand	6 65	1 13	7 78	5 53
J	San Joaquin loam	4 60	1 07	5.67	6 34
K	Sierra sandy loam	4.65	1.00	5 65	5.09
L	Yolo fine sand loam	7 35	2.21	9 56	9 11
M	Placentia loam	4 10	0 91	5 01	3 70
MG	Madera and Gridley loam	9 50	2.11	11 61	10 25
N	Okley fine sand	1.25	0.23	1 48	1.33
OC	Brockton clay	9 70	1 65	11 35	11 55
OL	Wooster silt loam	7 00	1 24	8 24	6.12
OS	Plainfield fine sand	1 50	0.29	1.79	1.36
S	Yolo silt loam	8 05	2.38	10 43	10.13
TL	Tehama loam	5 25	1.00	6 25	4.51
V	Catherine Loam	15 20	4 55	19.75	19 03
Y	Yolo fine sandy loam	6 90	2 21	9 11	8 93
YC	Yolo clay	12.2	3.67	15.87	13.98
AK_2	Aiken clay loam	13 30	3.10	16 40	20.75
YS	Yuma sand	2 95	0.78	3.73	3.17
Z	Brazito fine sand	1 25	0 40	1 65	1.58
24	San Joaquin sandy loam	4 32	0 88	5.20	3.93
25	Madera sandy loam	4 20	0 99	5.19	3 62
1	Davidson clay loam B	13 4	2 30	15 70	
2	McKenzie clay A	16.40	7.21	23.61	
3	Houston clay A	14 65	6.26	20 91	
4	Catalpa clay B	13 55	3.20	16.75	
5	Oktibbeha clay B	22.35	5 77	28 12	
6	Muck 1	53.70	15 20	68.90	
7	Muck 2	54.7	15.70	70.40	
8	Muck 3	54.0	14.60	68.60	

* Determined by Dr. F. J. Veihmeyer of the University of California.

with the direct method in which the plant was used as an indicator.[3] The results are shown in table 1.

An examination of the data reveals a remarkably close agreement between the wilting point as obtained by the two methods. It will be noted that in the majority of the soils the agreement is within 1 per cent. The only case in which the disagreement is considerable, 4.35 per cent, is in the Aiken clay loam.

DISCUSSION

A thorough consideration reveals no fundamental reason why the dilatometer method could not determine the wilting point because the wilting point represents a definite phase in the soil moisture range at which the pulling forces of the soil for the water commence to exceed the pulling forces of the plant for the same water. Now the pulling forces of the soil for the water are also exerted against the forces of crystallization or ice formation when the water freezes. At the temperature of −1.0°C., that portion of the water which is immediately influenced by the surface forces of the soil does not freeze and, if it freezes at lower temperatures, it remelts at −1.0°(1). This water which fails to freeze at −1.0°C. is definite in quantity for each soil and is the same as the wilting point.

It seems that the dilatometer method should be more accurate and more reliable in the general run of soils than the direct method for the following reasons:

The dilatometer tends to give absolute results because when the soil is frozen at −10°C. and then placed in −1.0°C. only that portion of the water remains frozen on which the soil exerts a tremendous influence through its adhesive and other forces. This unfrozen water at −1.0°C. is a definite value for each soil and is influenced mainly by the surface forces of the soil.

The wilting point as determined by the dilatometer method is not influenced by varied climatic factors.

In stickly clays and other soils the dilatometer should give more reliable results than the direct method because with the latter it is difficult to obtain uniform moisture throughout the soil column in which the plant grows.

The validity of the dilatometer method to determine the wilting point is further supported by the results of studies of the freezing point depression, as obtained by Bouyoucos (2), Veihmeyer (4), and Schofield (3).

SUMMARY

The dilatometer method is presented as a very rapid and accurate indirect method for determining the permanent wilting point of soils.

The dilatometer method has been compared with the direct method for determining the wilting point of soils, and the results show that the two methods agree closely.

[3] In this comparative study the writer is very grateful to Dr. F. J. Veihmeyer for furnishing him many samples of soils on which he had determined the permanent wilting point by the direct method, using the plant as an indicator.

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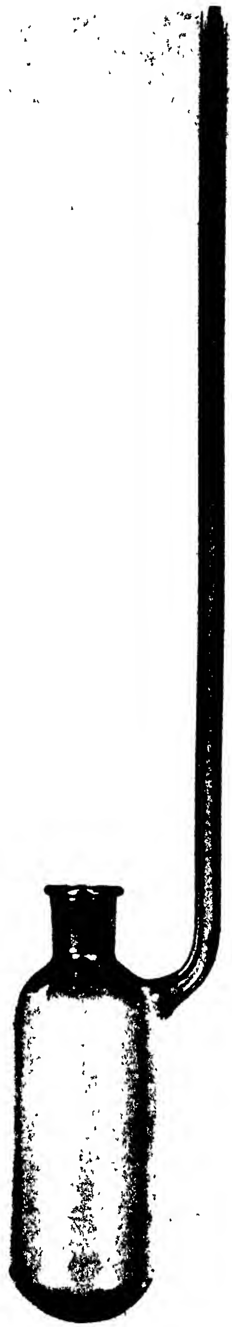
PLATE 1

Dilatometer Used in Determining the Wilting Point of Soils

This special dilatometer is manufactured by the American Instrument Company, Silver Springs, Maryland.



DIRECTIONS FOR MAKING MECHANICAL ANALYSES OF SOILS BY THE HYDROMETER METHOD[1]

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Since the hydrometer method is now so widely used for making mechanical analyses of soils, it has received further attention and study with the idea of possible improvement. As a result of further researches, the following improvements or changes have been made in the method:

A new type of dispersing machine (pl. 1) is now used, which is more convenient, less noisy, more durable, and more efficient than the original mixer.

A new hydrometer has been made which has a streamlined bulb, comes to equilibrium quicker, and is more accurate at the lower range of the scale than was the original hydrometer.

A new cylinder without lip is now used. This affords a better contact and consequently a better sealing between the palm of the hand and the mouth of the cylinder when the soil suspension is being shaken.

The cup has been improved in a few minor details.

Several minor changes in the technique add to the accuracy of the method.

The following is the latest procedure developed for making mechanical analyses of soils by the hydrometer method: Add 50 gm. of fine-textured soils or 100 gm. of the sand, based on oven-dry condition, to the dispersing cup. Fill the cup with distilled water to about 1½ inches of the top. Add to the contents 5 cc. of a solution of sodium silicate or water glass (2) having a hydrometer reading of 36, at 67°F., and 5 cc. of saturated and filtered sodium oxalate. If the soil is in lumps, sufficient time must be given to slake and to soak. As a matter of fact, it is well to allow all soils to soak for about 15 minutes before they are dispersed. The soils should always be air-dry, because in the wet condition they do not slake. The soaking can be done in a separate vessel and the material then washed into the cup. Connect the cup at the point where it is marked "attach here" to the stirring motor, and stir the contents for 5 minutes for sands and 10 minutes for all other soils. Those soils, however, which are recognized as difficult of dispersion should be dispersed for 20 or 30 minutes. The sands should not be stirred more than 5 minutes because they seem to undergo abrasion.

Pour and wash the contents into the special cylinder. If 50 gm. of soil is used, fill the cylinder to the lower mark with the hydrometer in it. If 100 gm. of soil is used, fill the cup to the upper mark with the hydrometer in it. Only distilled water should be used. Then take the hydrometer out, place

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the palm of one hand on the mouth of the cylinder, and shake the contents vigorously, turning the cylinder upside down and back several times. The palm when dry forms the best seal. Place the cylinder quickly on a stool or table and note the time immediately. At the desired period put the hydrometer in the suspension column, record the reading at the top of the meniscus, and then take it out again. Since there is a tendency for slight amounts of soil material to settle on the shoulder of the hydrometer, it is better not to leave it in continuously for all readings. Each time the hydrometer is used, it should be clean.

At every hydrometer reading, the temperature of the suspension should be measured. Great care must be taken, however, not to disturb too much the suspension column in putting in and taking out the hydrometer and the thermometer. For every 1°F. above or below 67°F., apply a temperature correction of 0.2 graduation on the hydrometer. This temperature correction, however, is only an approximation, as it tends to vary somewhat with extreme concentrations of soil suspensions and also with extreme variations of temperature. The most accurate hydrometer readings are taken near the temperature of 67°F., which is the temperature at which the hydrometer was calibrated in actual soil suspensions. Extreme temperatures such as 100°F. and 50°F. should be avoided. If possible, temperatures should be above 67° rather than below. For temperatures above 67°F., the corresponding amount of correction is added to the hydrometer reading, and for temperatures below 67°F., the corresponding amount is subtracted. The corrected hydrometer reading is then divided by the dry weight of soil taken and multiplied by 100, the result being the percentage of material still in suspension.

The hydrometer method cannot subdivide the sands into their various fractions but can only determine their combined or total amount. Experimental tests show that by taking a hydrometer reading at the end of 40 seconds the total combined sands of a soil can be ascertained almost to the same degree of accuracy as by the wet sieve method (sieve No. 270 with 0.053-mm. openings). A correct reading can be easily made in this short period except in some organic soils which have a tendency to produce froth at the top of the column after being shaken, in which case a correct hydrometer reading is not always possible. One way to overcome this difficulty is to add a drop or two of amyl alcohol after shaking. The procedure to be followed in determining the sands is as follows: After the soil has been shaden and the cylinder is placed on the table, a drop of amyl alcohol is quickly dropped at the top of the suspension column, one side of the cylinder wall is wiped clear so as to permit easy reading of the hydrometer, the hydrometer is placed into the suspension and steadied by hand, and at the end of 40 seconds the reading on the hydrometer is recorded.

Where a subdivision of the sands is required, the following procedure may be used: At the conclusion of the final hydrometer reading, the suspension is washed on a No. 270 sieve. That portion retained on the 270 sieve is dried

and analyzed on a set of sieves, consisting of one each of Nos. 20, 40, 60, 140, and 200.

The hydrometer was calibrated in actual soil suspensions consisting of an average loam soil. Its readings, therefore, are quite accurate.

A unique feature of the hydrometer method is the fact that the personal element is reduced to a minimum. The one important thing to watch is the condition of the paddle on the stirring rod. Practically the whole success of the hydrometer method depends upon a complete dispersion of the soil, and a complete dispersion can be obtained only when the paddle on the stirring rod or shaft is in good condition. The paddle that has been used heretofore in the method wears out very easily and becomes flat and small. In this condition its efficiency is practically gone, the soils are not properly dispersed, and consequently the results are not correct. In order to eliminate this serious weakness in the method, a new paddle or button has been adopted and placed on the stirring rod. When this paddle becomes worn it can be unscrewed from the shaft and replaced with a new one. This arrangement, besides providing a paddle that is wear-resistant, also obviates the necessity of changing the stirring rod in order to change the paddle, as has been the case heretofore. The paddles are inexpensive and can be changed frequently. It is strongly urged upon all those who are now using the old stirrer to replace it with the new one shown in plate 1.

Another important point is that the cup should have baffles in it, for without the baffles the soils can not be dispersed easily.

To calculate the conventional amount of combined sand (1.0–0.05 mm.), silt (0.05 – 0.005 mm.), and clay (0.005–000 mm.) and the finer clay (0.002–0.000 mm.) as determined by the hydrometer method, the procedure is as follows: The corrected hydrometer reading at the end of 40 seconds is divided by the amount of absolute dry soils taken and is multiplied by 100. The result is percentage of material still in suspension at the end of 40 seconds. This percentage is subtracted from 100, and the result is the percentage of material that settled out at the end of 40 seconds, which is supposed to represent all the sand in the soil. The corrected hydrometer reading at the end of 1 hour is also divided by the weight of soil sample and multiplied by 100. The result is percentage of material still in suspension and is considered to be the conventional clay (0.005–0.000 mm.). The percentage of the conventional silt is obtained by subtracting the clay from 100 and, from this result, subtracting again the percentage of sand. To obtain the finer clay (0.002–0.00 mm.) it is only necessary to divide the correct hydrometer reading at the end of 2 hours by the weight of the soil sample and multiply by 100.

From all studies and examinations thus far made, the hydrometer method appears to be reasonably accurate and reliable on all soils that are properly dispersed and properly stabilized by the reagent (1).

In peats and mucks, the hydrometer method is, of course, not applicable. In mineral soils containing a moderately high organic matter content, the

method is accurate provided the organic matter is well decomposed and is completely dispersed. Mineral soils containing a very high organic matter content in an incompletely decomposed state should be first treated with hydrogen peroxide.

In materials such as bentonite and fullers' earth the hydrometer method may not be very successful because these materials tend to gelatinize and emulsify and cause the hydrometer to stick and not to float normally and freely as it does in a true suspension.

It is advisable to wash very alkaline soils before using them for analysis, or a correction should be applied for the salts if present in appreciable amounts.

By taking hydrometer readings continuously or every so often, a complete distribution curve of size particles of soils and their respective amounts can be readily and reliably obtained (2).

Note: On account of the many inquiries received as to the price of the hydrometer method outfit, and where it may be obtained, the following information is given: The dispersing machine and cup (cost, about $22) are handled by the Hamilton Beach Co., Racine, Wis.; the hydrometer and cylinder (cost, about $5) are handled by the Taylor Instrument Co., Rochester, N. Y.

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PLATE 1

Improved Dispersing Machine, Hydrometer, Cup, and Cylinder, Used in the Hydrometer Method for Making Mechanical Analyses of Soils

MAKING MECHANICAL ANALYSES OF SOILS

GEORGE JOHN BOUYOUCOS

PHYSIOLOGICAL STUDIES ON RHIZOBIUM: VI. ACCESSORY FACTORS[1]

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The recent studies of Allison, Hoover, and Burk (1, 3, 9) concerning an accessory growth factor for Rhizobium have suggested a possible explanation for many difficulties encountered in the culture of these organisms. These workers reported that several species of the rhizobia were unable to make any appreciable growth in a synthetic (sugar-mineral-nitrate) medium prepared from highly purified materials. The failure to grow in such a medium was attributed to the absence of a factor essential for the respiration and growth of the organisms. They found a stimulative effect upon the rhizobia with additions of such substances as yeast extract, cane molasses, and humic acid and explained this as due to the presence of appreciable quantities of a factor which they designated as "coenzyme R."

Repetition of much of the work of Allison and Hoover, employing a medium similar to that used by those workers, has confirmed their results (12). Repeated attempts to culture various species of the rhizobia through several successive transfers in such a medium were unsuccessful if the sugar used was relatively pure.

Werkman (14) was able to culture *Rhizobium leguminosarum*, as well as several other organisms, however, through a large number of consecutive transfers in a wholly synthetic medium. He concluded that if accessory substances are needed by the organisms studied they are able to synthesize them.

The medium employed by Allison and Hoover (1) had the following composition: K_2HPO_4 – 0.7 gm., KH_2PO_4 – 0.3 gm., NaCl – 0.2 gm., $MgSO_4 \cdot 7H_2O$ – 0.2 gm., $CaSO_4 \cdot 2H_2O$ – 0.1 gm., distilled water – 1000 gm. The medium was filtered after standing 2 or more days, and 1 per cent sugar and sufficient KNO_3 to give 5 mgm. of nitrogen per 25 cc. of medium were added. For convenience this medium has been designated "medium A." The basal medium used by Werkman (14) differed in some respects from that of medium

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A. It was as follows: K_2HPO_4 — 2.0 gm., $MgSO_4 \cdot 7H_2O$ — 0.1 gm., $CaCl_2$ — 0.1 gm., $FeCl_3$ — trace, $(NH_4)_2SO_4$ — 2.0 gm., distilled water — 1000 cc. In the present investigation, this has been designated "medium B."

The reason for the different results obtained by Werkman and by Allison and Hoover was investigated by conducting a series of continuous culture experiments with the two media employed by the different workers. Sucrose c.p. was used as an energy source in both media. Experiments were conducted in 250-cc. Erlenmeyer flasks, each containg 25 cc. of medium. The media were inoculated in triplicate. The first inoculation was with a small quantity of culture from growth on yeast extract agar slants. At 4-day intervals the turbidity of cultures was observed, and transfers of 1 cc. of each culture were made into a similar medium. It was found that *Rh. meliloti*, *Rh. trifolii*, *Rh. japonicum*, *Bacillus subtilis*, and *Bacillus mycoides* were unable to continue growth through more than two or three transfers in medium A, but did continue to grow in medium B.

The composition of the two media differed principally in the nitrogen source and also in the presence of $FeCl_3$ in medium B. The addition of $FeCl_3$ to medium A greatly facilitated the growth of the organisms. The substitution of $(NH_4)_2SO_4$ for KNO_3 promoted much better growth of the organisms in the first few transfers. After that the organisms seemed to be adapted equally well to the two forms of nitrogen. In no case would they continue to grow, however, without the addition of a small quantity of available iron.

Allison and Hoover (1) found that the medium which they used contained usually enough iron as impurities to supply the needs of the rhizobia. In the present investigation several tests indicated, however, that the materials used in making the media did not contain enough iron to supply the needs of these organisms.

A review of the literature showed that ferrous and ferric iron have been very generally employed in bacterial media; but few indications were obtained as to which form was preferable for the organisms being cultured. The iron compounds most commonly used have been $FeSO_4$ and $FeCl_3$. An experiment was conducted to determine which of these compounds is most desirable for the rhizobia and also to find the optimum concentration of iron for their growth. Medium A, containing sucrose c.p. and KNO_3 (100 p.p.m. N) was employed as the basal medium. Ferric chloride and ferrous sulfate were added to the basal medium in the following concentrations of iron, in parts per million, for the different media: 0.2, 1.0, 2.0, 4.0, 20.0, 40.0, and 60.0. The basal medium, without any addition of iron, was used for the control cultures. The media were placed in 25-cc. portions in 250-cc. Erlenmeyer flasks and sterilized. The organisms employed for inoculum had been freed from iron and growth factors by repeated transfers in medium A containing commercial cane sugar as an energy source. Each flask of medium received 1 cc. of inoculum containing approximately 50 million organisms. Both *Rh. trifolii* and *Rh. meliloti* were used for the tests. The cultures were incubated 4 days with intermittent shaking. At the end of that time each culture was diluted to

50 cc. with sterile 0.1 N HCl, and direct microscopic counts were made employing a Petroff-Hausser bacteria counting chamber. In nearly every instance the triplicate cultures agreed closely in numbers of organisms. The average number of cells developing per cubic centimeter in each medium are shown for *Rh. trifolii* in figure 1. The results obtained for *Rh. meliloti* were closely similar to those shown in figure 1 except that the optimum concentration for $FeSO_4 \cdot 7H_2O$ was at 5 p.p.m. of iron.

Figure 1 and the data obtained for *Rh. meliloti* indicate that in optimum concentrations $FeCl_3 \cdot 6H_2O$ promotes a more rapid growth of *Rh. meliloti* and *Rh. trifolii* than does $FeSO_4 \cdot 7H_2O$. The optimum concentration of iron was 10 p.p.m. in all cases except the one noted. In concentrations of iron greater than 40 p.p.m. $FeSO_4 \cdot 7H_2O$ depressed the growth of both species of the organisms; in 60 p.p.m. of iron $FeCl_3 \cdot 6H_2O$ depressed the growth. Since these results indicated that the chemicals employed in making up the media contained insufficient iron to promote optimum growth of the organisms, $FeCl_3 \cdot 6H_2O$, in a concentration of 5 p.p.m. of iron, was employed in the media used in further investigations. In greater concentration the iron precipitated some of the constituents of the medium.

POTASSIUM NITRATE AS A NITROGEN SOURCE

Although iron greatly improved the growth of the rhizobia in medium A the addition of it did not enable the organisms to maintain growth when carried through repeated transfers in such a medium with sucrose c.p. as the energy source. It has been observed that a small quantity of inoculum of the nodule bacteria often fails to grow when transferred from a yeast extract agar slant into a KNO_3-sucrose c.p. medium. Allyn and Baldwin (4, 5) have shown that KNO_3 poises bacterial media at a potential so high as to be distinctly unfavorable for the growth of the rhizobia. From the data reported by these investigators it seems that other compounds employed as sources of nitrogen might promote better growth of the nodule bacteria than KNO_3.

The growth of various species of Rhizobium in a nitrate-sucrose medium was compared with their growth in media differing only in the nitrogen source employed. Three basal media were prepared containing the mineral salts of medium A (modified by the addition of $FeCl_3 \cdot 6H_2O$ in a concentration of 5 p.p.m. of Fe) with KNO_3, NH_4Cl, or asparagin, in concentrations of 100 p.p.m. of nitrogen, as the respective nitrogen sources. Two media were then made from each basal medium by the addition of sucrose c.p. and commercial cane sugar. The media were distributed in 25-cc. portions in 250-cc. Erlenmeyer flasks. The first inoculation was made with a small quantity of culture from yeast extract-agar slants. The liquid cultures were incubated at 28°C. with frequent shaking. At 4-day intervals the turbidity of the cultures was observed, and transfers of 1 cc. of each culture were made to a flask of similar medium. The relative growth, as indicated by the turbidity of the cultures, for several consecutive transfers is shown in table 1.

The data of table 1 show that *Rh. meliloti* and *Rh. japonicum* failed to make

TABLE 1

Continuous growth of rhizobia in medium A plus different sources of nitrogen and sucrose c.p. or cane sugar

TRANSFERS AT 4-DAY INTERVALS	RH. MELILOTI 132						RH. TRIFOLII 206					
	KNO$_3$		Asparagin		NH$_4$Cl		KNO$_3$		Asparagin		NH$_4$Cl	
	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.
1	+++						++++					
2*	+++	+	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
3	++	+	++++	++++	++++	++++	++++	+++	++++	+++	++++	++++
4	++	−	++++	++++	++++	++++	++++	++	++++	++++	++++	++++
5	+	−	++++	++++	++++	++++	++−	+	++++	++++	++++	++++
6†				++++		++++				++++		++++
7†				++++		++++				++++		++++

TRANSFERS AT 4-DAY INTERVALS	RH. LEGUMINOSARUM 306						RH. JAPONICUM 403					
	KNO$_3$		Asparagin		NH$_4$Cl		KNO$_3$		Asparagin		NH$_4$Cl	
	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.	cane	c. p.
1	++++								+++			
2*	++++	++++	++++	++++	++++	++++	+	+	++++	++++	++++	++++
3	++++	++++	++++	++++	++++	++++	−	−	++++	++++	++++	++++
4	+++	++	++++	++++	++++	++++	−	−	++++	++++	++++	++++
5	++	+	++++	++++	++++	++++	−	−	++++	++++	++++	++++
6†				++++		++++				++++		++++
7†				++++		++++				++++		++++

+ Indicates relative turbidity resulting from growth.

− Indicates lack of turbidity.

* Transfer 2 made from the first transfer in cane sugar and KNO$_3$ except in the case of *Rh. japonicum*, which was made from asparagin, cane sugar medium.

† In transfers 6 and 7 only the cultures in media containing c.p. sucrose and asparagin and ammonium chloride as sources of nitrogen were studied.

a good growth in the nitrate media. In no case, however, was there evidence of inability to grow in the asparagin or NH_4Cl media. The results also show that the four species of the rhizobia studied were able to maintain continuous growth in media composed of highly purified materials when asparagin or NH_4Cl was employed as the nitrogen source but were unable to do so with KNO_3 as the only source of nitrogen. The relative amounts of growth for only seven transfers are shown in table 1. Similar transfers were continued, however, in the asparagin and NH_4Cl media with sucrose c.p. as the energy source. After more than two months of continuous culturing there was no appreciable decrease in the growth rates of the organisms.

INFLUENCE OF REDUCING AGENTS

If the inability of the rhizobia to make appreciable growth in the nitrate-sucrose c.p. medium was due to an unfavorable potential produced by KNO_3 it is to be expected that the addition of reducing agents to the medium would greatly facilitate growth. Continuous culture experiments showed that both cysteine and thioglycollic acid, in optimum concentrations, greatly improved the ability of medium A to support the growth of the rhizobia. *Rh. meliloti* and *Rh. trifolii* produced good growth through several consecutive transfers in medium A with sucrose c.p. as the energy source if small quantities of cysteine or thioglycollic acid were added, but were unable to do so in the absence of these reducing substances.

Several experiments were conducted with *Rh. trifolii* to investigate the influence of cysteine upon oxygen consumption. The determinations were made with Warburg manometers. The organisms were prepared for inoculum by continuously culturing in KNO_3-cane sugar medium for several transfers and washing in a sterile solution of the mineral salts of medium A, one-half concentration. The Warburg manometeric technique, as given in previous articles of this series (11, 13), was employed. The oxygen consumed by the different cultures, together with the various modifications of the medium, are given in table 2.

The data of table 2 show that cysteine increased the rate of oxygen utilization of *Rh. trifolii* in media containing either KNO_3 or NH_4Cl as the nitrogen source. Assuming the change in rate of oxygen utilization of each culture for the consecutive hourly intervals as the criteria of growth, it is also seen that cysteine brought about significant increases in the growth of the organisms. A comparison of the oxygen consumed in the KNO_3 media containing cysteine and sucrose with that in the KNO_3-brown cane sugar medium shows that cysteine did not induce as great a stimulation of the organisms as might be expected from the accessory factors proposed by Allison and Hoover. In NH_4Cl media, however, the stimulative effects of cysteine were fully as great as those of brown cane sugar.

The results of the previous experiments indicate that the inability of the rhizobia to grow in the KNO_3-sucrose c.p. medium employed may be accounted

for in two ways: (*a*) The medium was deficient in iron; (*b*) potassium nitrate, in the concentrations used, poised the medium at a potential unfavorable for

TABLE 2

Oxygen consumed (cu.mm.) by Rh. trifolii during consecutive hourly intervals in medium A plus cysteine and different sources of nitrogen

Organisms for inoculum cultured in KNO_3-cane sugar medium

KNO_3 (100 P.P.M. N)						NH_4Cl (100 P.P.M. N)					
Sucrose (1 per cent) + Cysteine (per cent)					Brown cane sugar	Sucrose (1 per cent) + Cysteine (per cent)					Brown cane sugar
Control	0.0005	0.001	0.002	0.004		Control	0.0005	0.001	0.002	0.004	
13.0	14.5	13.4	15.7	13.8	16 3	14.2	15.2	16.4	16.9	16.1	17 9
12 6	14.5	14.7	14 6	13.7	18.3	14.5	15.5	15.9	15.9	15.3	17 3
13.1	14.4	14.6	15.0	13.8	22 9	14.8	15.8	16.1	17 5	15.3	19 3
13.3	15.9	15.3	16.3	13.0	27.6	15.4	16.4	16 4	19.0	17.4	20 6
13.0	17.0	15.2	16.0	13.2	28.2	15.8	15 8	16.9	20.4	17 1	20 4
13.0	17.7	15.7	17.7	13.7	38.2	15 4	16 4	17 8	28.3	20 8	24 4
12.9	17.9	15.5	17.4	13.2	47.8	15.9	16 9	17.7	31.2	21.4	26 2
10.6	20.8	16 5	16 4	9.8	58.4	16.4	16 6	16.6	36.1	23.1	27 7
11.8	27.2	15 0	17 7	10.5	68 7	15.4	17 4	17 5	45.6	28.1	30 0
10 6	34.5	13 1	19.6	10 2	60 6	16 0	17 0	18 2	52 8	31 5	35 3
12.1	42.1	16 1	24.8	10 7	58 6	16 0	19.0	20 2	46 9	39 6	40 2
14.1	51.1	20 3	31 5	14 0	57 0	20.9	21.9	25 9	43 4	40.5	47 2
19.0	56.6	28.6	36.3	17 6	49.9	27.3	27.5	31 6	33.1	44 5	52.0
169 0	344.2	214 8	259 0	167 2	552 5	219.2	231 4	247 2	407 1	330 7	378 5

TABLE 3

Numbers of Rh. trifolii after 2 days' growth in media containing various supplementary factors

(25 cc. medium inoculated with 15,000,000 organisms)

SUGAR AND SUPPLEMENTARY FACTOR	N SOURCE 100 P.P.M.	NUMBER OF ORGANISMS PER CC. (MILLIONS)		
		1	2	Average
Sucrose c.p.	KNO_3	6.06	6 64	6 35
Sucrose c.p. + sug. ext. 100 p.p.m.	KNO_3	258 40	250 80	254 60
Sucrose c.p. + sug. ext. 200 p.p.m.	KNO_3	288 80	303 60	296.20
Cane sugar	KNO_3	52.00	54.70	53.35
Cane sugar (recrystallized)	KNO_3	8 88	9.42	9.15
Brown cane sugar	KNO_3	214.40	208.40	211.40
Sucrose c.p. + *Az. vinelandii* med. 1–8	KNO_3	291.60	286.40	289.00
Sucrose c.p.	Asparagin	24.88	24.00	24.44
Sucrose c.p. + sug. ext. 100 p.p.m.	Asparagin	449.40	452.40	451.90

respiration and growth of these organisms. The data obtained seem to give no evidence that any accessory growth factor is essential for the growth or

respiration of the root nodule bacteria. It is recognized, however, that the accessory substances reported by Allison and Hoover (1) are able to induce large increases in the growth and respiration of Rhizobium in media composed of highly purified materials. This stimulative effect has been noted in media with NH_4Cl or asparagin, as well as KNO_3 as the source of nitrogen. A representative example of the stimulative effects of some of these accessory substances upon the growth of *Rh. trifolii* is shown in table 3.

The mineral salts solution of medium A was used as the basis for the various

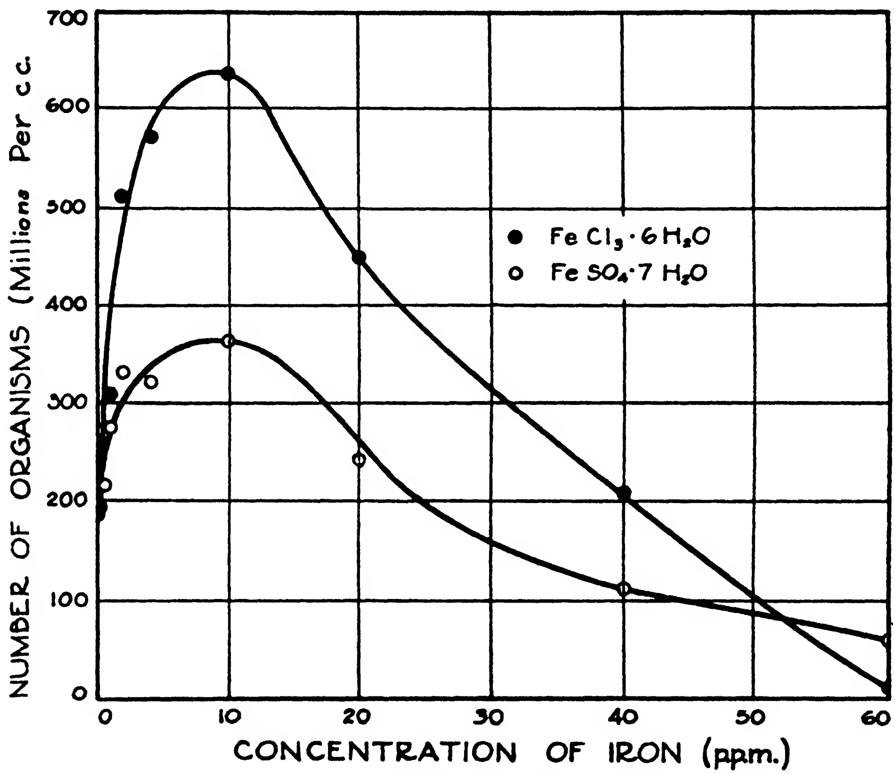

FIG. 1. GROWTH OF RHIZOBIUM TRIFOLII IN MEDIA CONTAINING FERROUS AND FERRIC IRON IN DIFFERENT CONCENTRATIONS

media shown in table 3. The organisms used for inoculum had been freed of accessory factors by culturing in nitrate-cane sugar medium. The culturing of the organisms and the direct microscopic counts were performed similarly to the procedure outlined in connection with figure 1. The sugar extract was prepared by the extraction of commercial cane sugar with absolute alcohol in the manner described by Allison and Hoover (1). A portion of the cane sugar was recrystallized from an 80 per cent solution of alcohol. The *Az. vinelandii* medium was prepared by centrifuging the organisms from a 3-week-old culture of *Az. vinelandii* growing in medium A with sucrose c.p. as

the energy source. This medium was added in a concentration of one-eighth by volume to the medium used in the test.

The data of table 3 show that the growth of the organisms was increased by the addition of the various accessory materials in just about the same degree as that reported by the previous workers. Cane sugar extract induced a 40- to 50-fold increase in the number of organisms in the KNO_3 medium and about a 20-fold increase in the asparagin medium. Studies with Warburg manometers have shown that these materials bring about 2- to 5-fold increases in the oxygen utilization of the organisms in similar media during an 8-hour period.

Some investigations have been conducted to determine the distribution and relative effects of such accessory substances. Medium A with sucrose c.p. as an energy source was used in most of the determinations. In some cases NH_4Cl or asparagin was employed as the source of nitrogen in place of KNO_3.

A water extract of alfalfa was fractionated into four parts on the basis of solubility of the parts in water-alcohol solutions of different concentrations according to the procedure of Fulmer, Duecker, and Nelson (6). The fraction soluble in 70 per cent but insoluble in 95 per cent alcohol and the fraction soluble in 95 per cent alcohol had similar stimulative effects upon the rate of oxygen utilization by *Rh. trifolii* and *Rh. meliloti.*

Water extracts of several different soils promoted an increased rate of oxygen uptake by the species of Rhizobium studied. In general the extracts of soils of high fertility were more stimulative than extracts of soils of low fertility. It was also found that heating sucrose c.p. with a solution of K_2HPO_4 or KH_2PO_4 resulted in the formation of a stimulant for the organisms.

On the basis of the preceding results it is difficult to accept the opinion that the beneficial effects of the various accessory substances studied were due to the presence of varying amounts of one specific substance. In spite of the variable nature of the stimulative agents investigated the problem has many parallels in the identification of such materials as growth factors, hormones, vitamins, and auxins for animals and plants. For this reason it is not improbable that certain groups may be found which are able to induce all of the responses in the rhizobia which have been brought about by the various accessory substances investigated.

DISCUSSION

In a recent paper, Allison and Hoover (2) reported that the addition of available iron to the medium used induced usually only a very small increase in the growth of the rhizobia. The stimulative effect of additions of ferric citrate or ferric sulfate was usually less than 10 per cent. The medium used in the work of these investigators was similar to that employed in the present investigation, except that the sugar which they used was, according to their description, a commercial sucrose containing considerable amounts of coenzyme R. Sucrose c.p. was employed in the present study. The different

materials used in the media of the two investigations make it quite probable that different quantities of iron were present as impurities. As a result, the responses to iron additions would be different. The data from this investigation indicate that although iron is essential for the growth of Rhizobium it could hardly be classed as a growth stimulant. The results of Allison and Hoover (2) do not appear to be in disagreement with this conclusion.

The use of the term "coenzyme R" has not been adopted in this paper, since no evidence has been obtained which shows that such a substance is essential for the respiration or growth of the rhizobia. Furthermore, it is felt that the term "coenzyme," as evidenced by the discussions of Hackh (7), Harden (8) and Kluyver (10), has no well-established meaning. In view of the conflicting opinions the more general terms "accessory growth substances" and "growth factors" have been employed.

SUMMARY

The addition of small quantities of iron to the nitrate-sucrose c.p. medium employed greatly increased the growth of *Rh. trifolii* and *Rh. meliloti.* Ferric chloride was found to promote greater growth of these organisms than ferrous sulfate. The optimum concentration of iron in the medium was found to be about 10 p.p.m.

Several species of Rhizobium were unable to maintain growth through repeated transfers in a mineral salts-sucrose c.p. medium with KNO_3 as a nitrogen source. If NH_4Cl or asparagin were employed as the source of nitrogen in place of KNO_3 the organisms could be continuously cultured in such a medium for indefinite periods of time.

The addition of reducing agents such as cysteine or thioglycollic acid increased the growth and oxygen utilization of the rhizobia in media composed of highly purified materials.

No evidence was found which indicates that the root nodule bacteria require any complex, unidentified substances for their growth. Many materials, however, are able to stimulate the growth and respiration of these organisms in mineral salts-sucrose c.p. media with KNO_3, NH_4Cl, or asparagin employed as sources of nitrogen.

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Joseph N. Harper

Joseph N. Harper
1874-1936

Dr. Joseph N. Harper, for many years identified and widely known in connection with soil fertility interests in the South, passed away at his home at Atlanta, Georgia, July 1. His many friends who have known of his poor health due to a heart ailment, nevertheless were sadly shocked, since he had been active up to within a few days of his death.

Few agriculturists in the South have been more closely allied with its soil fertility problems than was Joseph Harper. Born March 11, 1874, and reared on a farm in Winston County, Mississippi, he began his advisory career with his appointment in 1898 as agronomist to the Kentucky Agricultural Experiment Station where he made notable contributions on the culture of tobacco, corn, wheat, and hemp.

In 1904 he installed at the St. Louis fair a Kentucky agricultural exhibit which won a grand prize and many medals and led to a special invitation from the Department of Agricultural Technology of Ireland to visit that country and conduct experiments with tobacco. While abroad, Doctor Harper studied soils and methods practiced by farmers in maintaining soil fertility in the British Isles.

In 1905 he was called to head the department of agriculture of Clemson College, South Carolina, and to direct the activities of the South Carolina Experiment Station. These positions he held for 11 years, and under his direction the research department of Clemson College grew until it became recognized as a leading experiment station dealing with problems of soil fertility and plant diseases.

Doctor Harper, in 1917, was chosen to direct the extensive work of the Soil Improvement Committee of the Southern Fertilizer Association. His sound scientific knowledge and practical judgment won for him, in his travels all over the South, the respect of all concerned with the maintenance and building up of soil fertility.

After a year as director of the department of crop fertilization of W. R. Grace & Co., in 1925 Doctor Harper accepted a position as director of agricultural research for the Société Commerciale des Potasses d'Alsace with headquarters in Atlanta. With the formation of N. V. Potash Export My., Inc., Doctor Harper became a director of this company's agricultural and scientific bureau, in charge of the southern territory, which position he held until the formation of the American Potash Institute, Inc., in July, 1935. For the institute he was manager of the southern territory.

Doctor Harper held memberships in many scientific societies and had held every office in the Association of Agricultural Workers, which is composed of the leading agricultural scientists of the South. It has been said of him that his success was due not only to his scientific knowledge, but to his practical knowledge of farming, and that when he talked to farmers he had his own experience of a lifetime of farming from which to draw.

He is survived by Mrs. Harper, whom as Miss Susan Sparks he married in 1898, and by one daughter, Lucy Elizabeth.

R. H. Stinchfield.

PROPERTIES OF THE HYDROXYL GROUPS OF CLAY AS A BASIS FOR CHARACTERIZING A MINERAL SOIL[1]

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INTRODUCTION

By the conductometric titration of a soil treated with increasing concentrations of $Ba(OH)_2$, Hissink (17, 18) found that the salt line representing the formation of the barium salt of the soil acids coincided with the abscissa on which were plotted the quantities of $Ba(OH)_2$ added, indicating saturation of these acid functions. It has been found also by Hissink and later by others that the conductivity of the solution at the points of the straight portion of the baryta line is proportional to the content of baryta in solution. Hissink accordingly proposed that the degree of unsaturation of a soil represented by his quantity $T - S$ (T = maximum absorbing capacity, S = replaceable bases) could be determined by the point represented by the intersection of the straight portion of the baryta line with the abscissa. Hissink pointed out that, although the method was not free of objection, the horizontal portion of the bartya line corresponded approximately to the so-called "replaceable" hydrogen ions. But a number of investigators (2, 20, 21, 24, 26) have pointed out that Hissink's quantity ($T - S$) is much higher than that corresponding to the replaceable hydrogen ions.

The resulting confusion appears to have delayed the recognition of the possibilities of the acid and base titrametric method as a means for the rapid characterization of soils. The principal objection which has been raised is that secondary reactions take place—that the barium (or calcium) hydroxide reacts not only with the base exchange components but also with other constituents of the soil—when *an excess* of alkali or alkali hydroxides is added to a soil (21, 25).

On the other hand, by treating soils with increasing concentrations (0.005 N to 0.04 N per gram of soil) of $Ba(OH)_2$ in aqueous solution and also of KOH in alcoholic solution both before and after leaching with a solution of 0.05 N HCl, Clarens (12) has *established the existence of a gamut of acidic functions in a soil, the barium salts of one of which are decomposed by carbonic acid but not by water.* The existence of such unsaturated functions was established even in soils containing as high as 6.6 per cent $CaCO_3$. From these experiments

[1] Authorized for publication on June 18, 1936, as Paper No. 733 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

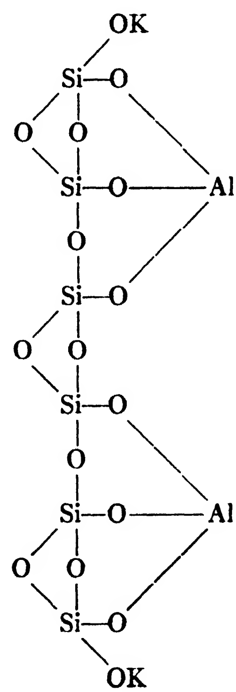
Clarens (5, 6, 9, 11, 12, 13) developed a method for the rapid characterization of a soil which the writer made use of on being confronted with difficulties in the interpretation of some of the results obtained in investigations on the condition of the phosphorus and also of the potassium in apple tree cylinder experiments (28, 31) by methods of continuous extraction with different dilute acids (30, 31).

The concept developed is essentially one that is at present generally accepted; namely, that the dominant exchange complex in soils of mineral origin consists of a series of alumino-silicic acids containing hydrogen ions of different activities (reaction values) (1, 3, 15, 24).

The constitution of the feldspars, kaolins, and clays

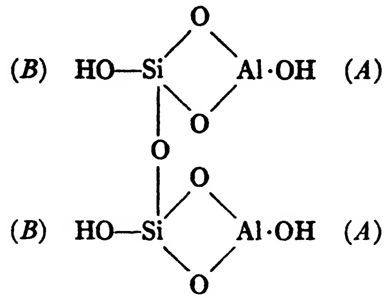
The constitutional formulas of the feldspars, kaolins, and clays represented below are not given as final but as indicating the convergence of our present knowledge. They serve as a means to the interpretation of the results of our experiments.

Basing our concepts of the constitution of the silicates of aluminum given by Clarke (14), we may deduce the structural formula of an unmixed orthoclase feldspar ($K_2Al_2Si_6O_{16}$)



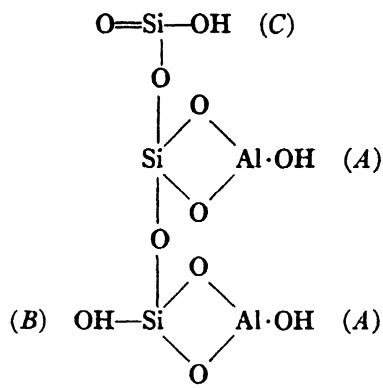
from one of the ortho-silicic acids (H_4SiO_4), by the elimination of 8 molecules of water from 6 molecules of H_4SiO_4 and the substitution of 6 hydrogen by 2

aluminum atoms and 2 hydrogen by 2 potassium atoms; and the constitution of a pure kaolin ($H_4Al_2Si_2O_9$)

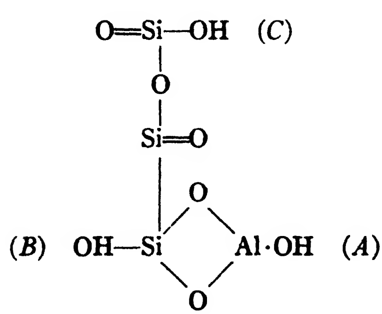


from orthoclase feldspar by the elimination of 4 molecules of SiO_2 and 2 of K.

In accordance with current concepts of the constitution of clay; namely, that clays are compounds of a series of alumino-silicic acids and feldspars, we may then deduce the structural formula for clay as typified by the following constitutional formulas:



or (9)



The three types of hydroxyl groups of clays

It is to be noted that the hydroxyl groups marked (*C*) in the formula for clay are not present in kaolin. Experimental evidence in support of this will

be given later. Since these OH groups denoted by (*C*) are attached directly to Si atoms not directly linked to the Al atoms, they will possess distinct acidic properties—functioning as hydrogen silicate—and can be replaced partially or wholly by different metals, such as K, Ca, Mg.

$$O{=}\overset{|}{\underset{|}{Si}}{-}OH + KCl = O{=}\overset{|}{\underset{|}{Si}}{-}OK + HCl$$

and, conversely,

$$O{=}\overset{|}{\underset{|}{Si}}{-}K + HCl = O{=}\overset{|}{\underset{|}{Si}}{-}OH + KCl$$

Some of these hydroxyl groups marked (*C*) are free, and a portion of them may be replaced by bases. The free hydrogens can be determined by the quantity of $Ba(OH)_2$ *neutralized* in the formation of barium salts; and the hydrogens replaced by metals, by the quantity of HCl required to effect this substitution.

The hydroxyl groups marked (*B*) are linked to the Si atoms connected to Al atoms by an oxygen linkage. The hydrogens of these groups marked (*B*), therefore, will possess less pronounced acidic properties than those marked (*C*). With baryta the neutralization of the hydrogens of the OH groups marked (*B*) is only partial, since (*vide infra*) the barium salts of these acidic functions are hydrolyzed by water.

The hydroxyl groups denoted by (*A*) are linked directly to Al, an electropositive element with basic properties. The salts obtained by the substitution of acid radicals in these OH groups undergo partial hydrolysis. The total replacement of these hydroxyl groups marked (*A*) can be obtained only when an excess of acid is used. When treated with an acid the amount of the acid radical fixed serves to evaluate the number of these hydroxyl groups substituted (*vide infra*)

$$\overset{|}{\underset{|}{Al}}{-}OH + HCl = \overset{|}{\underset{|}{Al}}{-}Cl + H_2O$$

MATERIALS AND METHODS

The original soil and also soils from two of the metal cylinders were used in these experiments. The soil designated as the "original" soil is the soil in its initial state before being introduced into the cylinders. It is a virgin soil of the Hagerstown series and has been fully described in an earlier publication (27). The soils designated "NPK" and "check" are representative of the samples of soils taken at the end of the experiment in the manner already described (29) from the surface 0–7 inches of the NPK treated and untreated cylinders under cultivation.

To 1-gm. portions of soil (sieved to pass a 100-mesh screen) in two series of tubes (from eight to ten tubes in each series) were added, to the one series,

increasing quantities of 0.1 N HCl and, to the other, increasing amounts of 0.1 N $Ba(OH)_2$ in the following manner: To one tube 19 cc. of water and 1 cc. 0.1 N HCl (or 0.1 N $Ba(OH)_2$), to a second 18 cc. of water and 2 cc. of 0.1 N HCl (or 0.1 N $Ba(OH)_2$), and so on for eight to ten tubes. The volume of the solution in each tube is accordingly 20 cc. Freshly boiled distilled water was

TABLE 1

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the "original" soil with increasing amounts of the acid and base

cc. 0.1 N HCl			cc. 0.1 N $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0.20	0.80	1	0	1.00
2	1.05	0 95	2	0 30	1.65
3	1.90	1.10	3	1.10	1.90
4	2.75	1.25	4	1.90	2.10
5	3.60	1.40	5	2.70	2.30
6	4.55	1.45	6	3 65	2.35
7	5.55	1.45	7	4 65	2.35
8	6.55	1 45	8	5.65	2.35
9	7 40	1 60	9	6 50	2.50
10	8.30	1 70	10	7.35	2.65

TABLE 2

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the check soil with increasing amounts of the acid and base

cc. 0.1 N HCl			cc. 0.1 N $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0 15	0.90	1	0	1 00
2	0 85	1 20	2	0 25	1 75
3	1 55	1.45	3	1 05	1.95
4	2.50	1 50	4	1.90	2.10
5	3 45	1.55	5	2.80	2 20
6	4.35	1.65	6	3.75	2.25
7	5.35	1.65	7	4 75	2.25
8	6.10	1.90	8	5.60	2 40
9	6.95	2.05	9	6.35	2.65
10	7.80	2.20	10	7.15	2 85

used. After leaving the soil in contact with the reagent for 18 hours, the tubes were continuously shaken for 6 hours. On settling, the solutions were filtered with suction with proper precautions in the case of $Ba(OH)_2$, and the number of cubic centimeters of 0.1 N HCl remaining in an aliquot (10 cc.) was titrated with methyl orange as indicator against standard alkali. The 0.1 N $Ba(OH)_2$

remaining in solution was titrated with phenolphthalein as indicator against standard acid.

For reasons the significance of which is pointed out later in this paper, the

TABLE 3

Amounts of 0.1 N HCl and of 0.1 N $Ba(OH)_2$ remaining in solution after treatment of the NPK soil with increasing amounts of the acid and base

cc. 0.1 *N* HCl			cc. 0.1 *N* $Ba(OH)_2$		
Added	Remaining in solution	Fixed	Added	Remaining in solution	Fixed
1	0.10	0.90	1	0	1 00
2	0.70	1.30	2	0.20	1 80
3	1.30	1.70	3	0.95	2.05
4	2.20	1.80	4	1.65	2.35
5	3.10	1 90	5	2.45	2.55
6	3.95	2.05	6	3.35	2 65
7	4.85	2.15	7	4 35	2.65
8	5.65	2.35	8	5.35	2.65
9	6.45	2.55	9	6 25	2.75
10	7.20	2 70	10	7.15	2.85

TABLE 4

Amounts of 0.1 N NaOH required to satisfy all bases remaining in solution, including Al and Fe, after treatment of the soils with increasing amounts of 0.1 N HCl

(Phenolphthalein titration of boiling solutions following the titration with methyl orange)

ORIGINAL		CHECK		NPK	
0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution	0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution	0.1 *N* HCl added	0.1 *N* NaOH required to neutralize all bases remaining in solution
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	0.50	1	0.50	1	0.50
2	1.50	2	1.40	2	1.30
3	2.45	3	2.30	3	2.15
4	3.50	4	3.30	4	3 15
5	4 50	5	4.40	5	4.10
6	5.55	6	5.45	6	5.10
7	6 60	7	6 55	7	6.10
8	7.60	8	7.55	8	7.10
9	8.45	9	8.40	9	8.00
10	9.35	10	9.30	10	8.90

titration of the HCl remaining in solution is made first with methyl orange in the cold. The solution is then heated to boiling, and after the addition of a few drops of phenolphthalein the titration is continued to a permanent pink color.

EXPERIMENTAL DATA

The results are given in tables 1 to 4 and are shown graphically in figures 1 to 3.

The "neutralization" portion of the graphs obtained for a particular soil consists of three segments; the equilibrium points making up each of the segments lie approximately on a straight line, the deviations being relatively small. The first segment is horizontal and, therefore, coincides with the abscissa; a

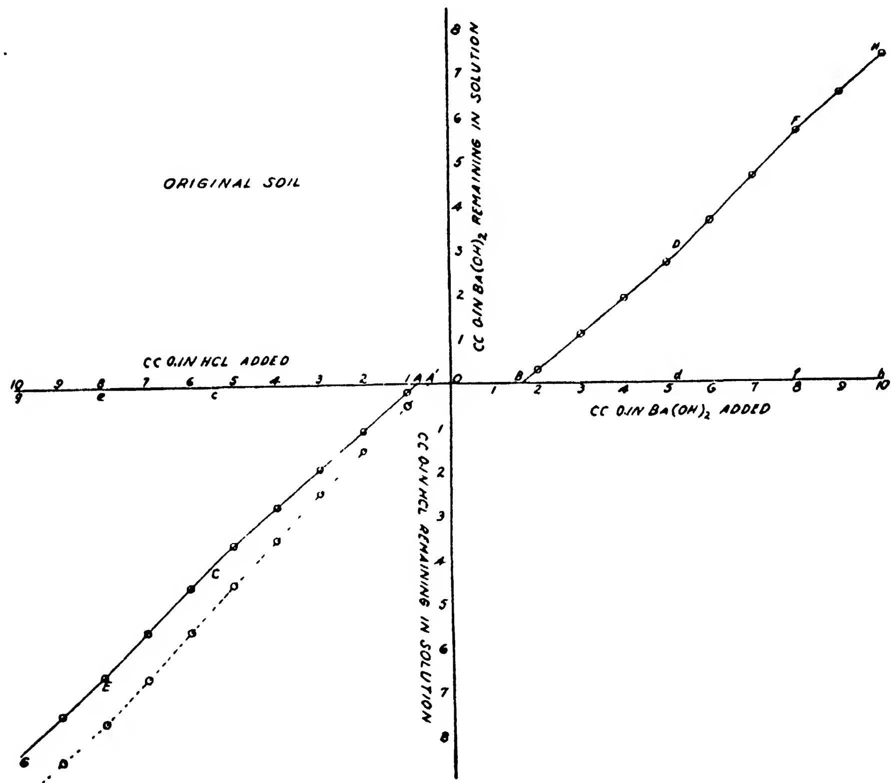


FIG. 1. THE "ORIGINAL" SOIL TREATED WITH SOLUTIONS OF 0.1 N HCl AND OF 0.1 N $Ba(OH)_2$

second segment makes an angle of less than 45°; and a third segment has an angle the tangent of which is approximately unity, indicating saturation at the higher concentrations.

DISCUSSION OF RESULTS

The interpretation of the graphs

The results for the "original" soil, represented in figure 1, are described in detail to illustrate the method of interpretation.

The segment OB. In the "original" soil OB is equal to 1.62. This indicates that complete fixation of the baryta added has occurred up to a concentration of 1.62 cc. 0.1 N $Ba(OH)_2$ per gram of soil. The segment OB then evaluates those acidic functions the barium salts of which are not hydrolyzed by water free from CO_2 and corresponds to the unsaturated part of the hydroxyl groups denoted by (C) in the formula for clay previously given. Even in soils containing large amounts of $CaCO_3$, the segment OB will not be zero because

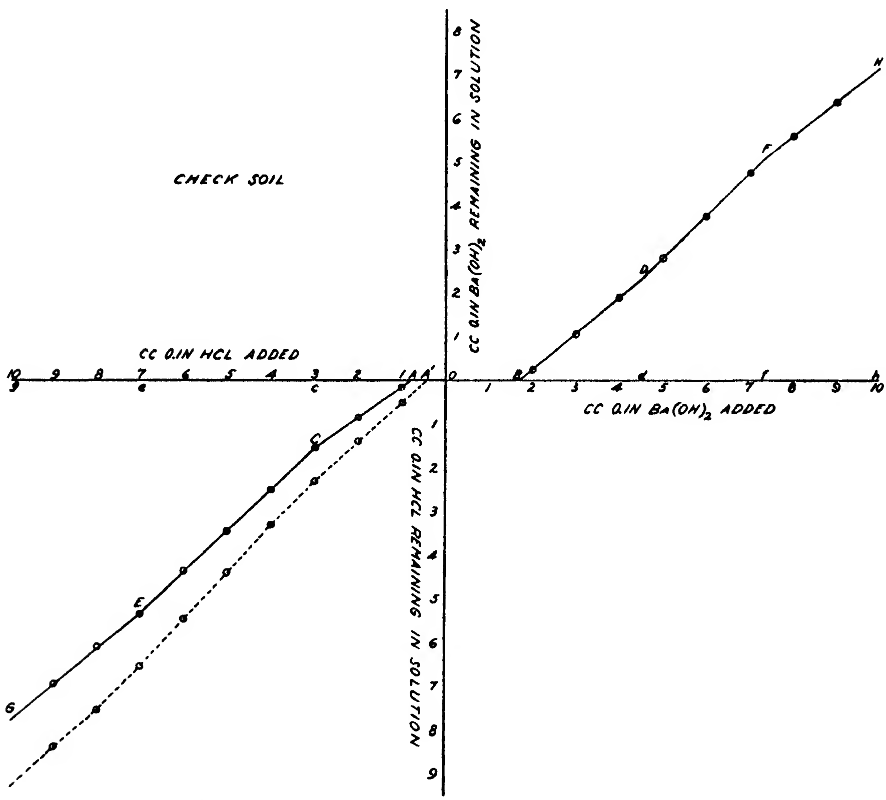


FIG. 2. THE "CHECK" SOIL TREATED WITH SOLUTIONS OF 0.1 N HCl AND OF 0.1 N $Ba(OH)_2$

of the presence of $Ca(HCO_3)_2$ in the soil solution. OB is only fully saturated by $Ba(OH)_2$ or, therefore, by $Ca(OH)_2$ when a slight excess of alkali is present.

The segment BD. After the replacement (neutralization) of the hydrogens represented by the segment OB, there still exist acidic functions the barium salts of which are partially hydrolyzed by water. This segment BD corresponds to those hydroxyl groups marked (B) and makes an angle—tan $D_Bd = 0.82$. This indicates that after a quantity of 0.1 N $Ba(OH)_2$ equal to OB (1.62 cc. per gram of soil) is added, the fixation of this base is only 18 per cent. The segment BD is evaluated thus: $Bd - Dd = 3.40 - 2.70 =$

0.70 cc. 0.1 N $Ba(OH)_2$ per gram of soil. The point d represents the intersection of the perpendicular from D to the abscissa. The acid functions represented by BD are of no practical importance, since the complete saturation of these groups would necessitate such an excess of base as would make the soil unfit for plants to thrive.

The segment DF. Commencing at D the third segment, DF, makes an angle of approximately 45° with the abscissa, indicating that the fixation of the base along this segment is nil, with formation of barium salts corresponding to other

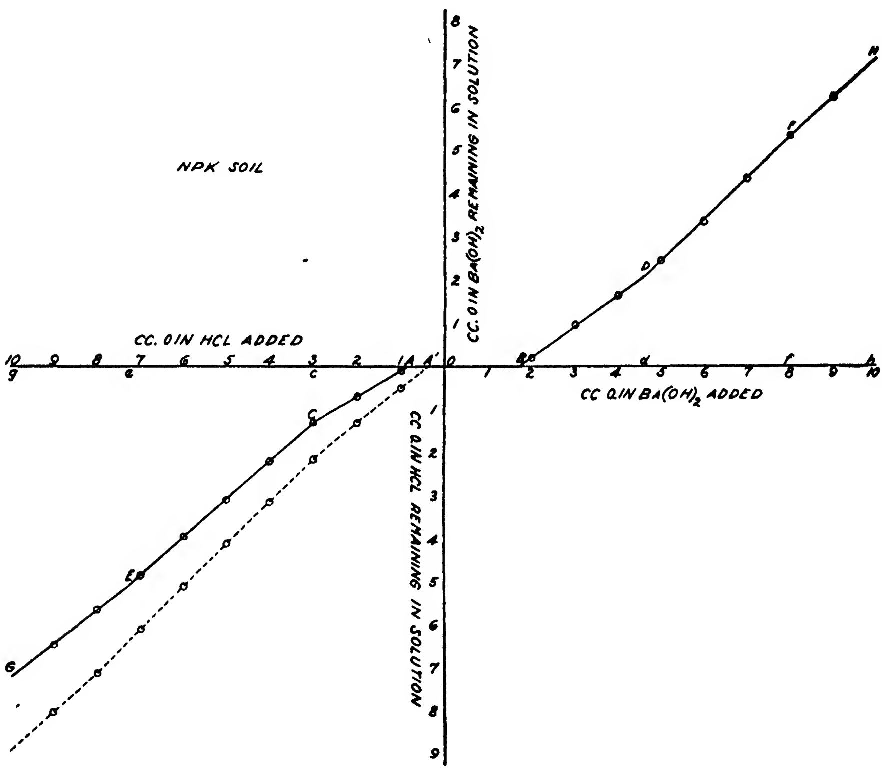


FIG. 3. THE "NPK" SOIL TREATED WITH SOLUTIONS OF 0.1 N HCl AND OF 0.1 N $Ba(OH)_2$

acidic functions having no affinity for $Ba(OH)_2$. Above a concentration of 8 cc. 0.1 N $Ba(OH)_2$ the curve again breaks and makes with the abscissa an angle the tangent of which is less than that made by DF. This segment FH has no bearing in these experiments. It is an indication of the instability of the clay complex, since above this concentration the baryta, instead of neutralizing, reacted with some of the components of the clay.

The segment OA. In the "original" soil OA is equal to 0.76 cc. In the absence of free bases, such as $Al(OH)_3$, $Fe(OH)_3$, $CaCO_3$, this segment would represent the number of cubic centimeters of 0.1 N HCl per gram of soil re-

quired to react with the exchangeable bases. OA then represents that fraction of the hydroxyl groups marked (*C*) the hydrogens of which have been replaced by metals.

If kaolin is substituted for soil in these experiments the points O, A, and B almost coincide (9). Even when digested with 1.7 *N* HCl for 10 hours in a boiling water bath, no decrease in the exchange capacity of either dickite or kaolin occurred (15). The segment AB, therefore, is relatively insignificant in kaolin, evidence that the hydroxyl groups marked (*C*) have, therefore, no equivalent in kaolin. The remainder of the graphs for kaolin do not differ from that for soils, i.e., there is a segment AC making an angle of less than 45° and a third segment the tangent of which is unity.

Although in the case of most of the soils examined by Clarens, free aluminum and iron hydroxides were absent, this is not the case with the Hagerstown soils reported here. The behavior of this Hagerstown clay loam to very low concentrations of HCl (0.000182 *M* to 0.00182 *M*) is intermediate to the behavior of kaolin and that of feldspar.

Whereas in kaolin the segment OA is insignificant, in feldspars the whole of this segment OA is represented by Al (9). An unmixed feldspar, examined by the writer, treated with increasing quantities of 0.1 *N* HCl titrated against 0.1 *N* alkali, using as indicators methyl orange followed by phenolphthalein, as described, subsequently, gave OA = 2.6 cc. 0.1 *N* NaOH per gram of feldspar and OA′ = 0.05. AA′, therefore, is equal to 2.55, showing that practically all of the exchangeable bases in this feldspar are represented by Al. The segment OB for feldspar was found to be 0.15 and, therefore, practically negligible.

It is evident then that the segment OA represents the exchangeable bases only if the soil contains no free bases or carbonates that may react with the acid employed. In the soils reported here the carbonates are relatively low (0.05 per cent), but appreciable quantities of "free" aluminum (and iron) are present. The fraction of the segment OA to be assigned to Al_2O_3 and Fe_2O_3 is represented in the figures by AA′ and can be obtained by titration with 0.1 *N* NaOH first at room temperature with methyl orange as indicator followed by titration of the solution raised to boiling temperature using phenolphthalein as indicator. A solution of $AlCl_3$ (or $FeCl_6$) neutral to methyl orange is acid to phenolphthalein. In titrating with 0.1 *N* NaOH starting from the point of neutralization with methyl orange, the phenolphthalein turns color only when all the aluminum and iron are precipitated as hydroxides. The difference between the two titration values with these indicators gives approximately, therefore, the quantity of $Al(OH_3)$ (and $Fe(OH)_3$) in solution in the form of $AlCl_3$ (or $FeCl_3$). The broken lines (fig. 1–3) and table 4 give the results with phenolphthalein as indicator.

In the "original" soil AA′ = 0.26 cc. gives the Al (and Fe) replaced per gram of soil. The exchangeable bases are represented by OA′ = 0.50 cc. 0.1 *N* HCl per gram of soil. In order to determine OA in calcareous soils, it is neces-

sary to ascertain the quantity of 0.1 N acid necessary to neutralize the calcium carbonate in 1 gm. of soil. This quantity is then subtracted from the value obtained for the segment OA.

The segment AC. In the "original" soil the second segment AC makes an angle tan cAC = 0.84, indicating that the HCl has been fixed to the extent of 16 per cent. The value of AC is given by Ac − Cc = 0.7 cc. 0.1 N HCl per gram of soil, where c is the perpendicular from C on to the abscissa.

The acidic functions represented by the segment AC cannot be replaced by $Ba(OH)_2$. This has been repeatedly established (9) by treatment of soils which have previously been treated with increasing quantities of 0.1 N HCl in the manner already indicated, with increasing quantities of 0.1 N $Ba(OH)_2$ in amounts more than sufficient to effect complete substitution of all the hydrogen atoms. In all cases the hydrogen replaced by barium was always expressed by the value of the segment AB before the initial treatment with HCl. The segment AC then must correspond to the hydroxyl groups marked (A) in direct attachment with Al. Relative to the origin of the aluminum, Clarens states (10)[2]:

> This aluminum could have prevously existed in the free state or even have come from new silicates, which may either have remained attached to the rest of the molecule, so that spaces left by the aluminum might be filled by a metal of some sort, or have been carried off themselves in the form of SiO_2. The importance of these alternatives to the fate of feldspar that has become clay, or more accurately zeolite, is apparent.
>
> . . . It is evident that the feldspar which we have just been studying will not be transformed into zeolitic clay by the usual means (replacement of the alkaline and alkaline earth bases by various agents, particularly by H_2CO_3). It is the aluminum which will first be replaced, according to circumstances, by various bases which, in the resulting zeolitic clay, will constitute the replaceable bases; a small, usually very small, amount of the aluminum may perhaps remain and also act as a replaceable base. It is the potassium thus fixed that is called the "assimilable" potassium of the clay.

The segment CE. A third segment, CE, making an angle of 45° indicates that the acid functions represented are saturated and that no further fixation takes place.

On the addition of more acid the curve again breaks and makes an angle of less than 45°. Inasmuch as this indicates an attack on the clay nucleus, it is of no interest in these experiments.

The three segments of importance in application. The most important segments are OA, OB, and AC. OA evaluates, in the absence of free aluminum and ferric hydroxides and calcium carbonate, the acidic functions actually saturated by the so-called "replaceable bases"; OB evaluates free acidic functions the barium salts of which are not hydrolyzed by water free from CO_2 in the presence of a slight excess of base. The segment AC bears the same relation to acids, in particular to phosphoric acid, as the segment BD does to bases. This fact may be used to determine the amount of phosphoric acid

[2] Translation from the original French.

necessary to satisfy the Al and Fe. It should be apparent that only four points are required to obtain all three segments.

Application to the determination of the amount of phosphoric acid required to satisfy the Al and Fe

In the "original" soil the angle which AC makes with the abscissa (tan = 0.84) indicates (assuming the fertilizer to be mixed thoroughly with the soil, which is never the case in practice) that if less than 0.84 cc. 0.1 N phosphoric acid per gram of soil be added the whole of the phosphoric acid would be fixed, resulting in a low concentration of phosphorus in the soil solution until this "threshold value" is exceeded.

Calculated as P_2O_5, the threshold value for the "original" soil is equal to

$$0.84 \times 0.002358 \times 585130 = 1159 \text{ gm. } P_2O_5$$

where 0.002358 is the number of grams of P_2O_5 in 1 cc. of 0.1 N acid and 585130 is the weight of the soil (in grams) in the 0 to 7-inch layer. Eleven hundred and fifty-nine grams of P_2O_5 is then required to saturate the segment represented by AC of the 0 to 7-inch layer in the "original" soil. Calculated to an acre basis of 2,000,000 pounds the value obtained is 3957 pounds P_2O_5, which is relatively enormous. In practice, the phosphate fertilizer is not intimately mixed with the whole of the first 7 inches. Under field conditions the diffusion of phosphate is very slow and frequently extends over a long period, thus allowing portions of the soil to be in contact with high concentrations of phosphate, and for this fraction the limiting value above is passed.

Application to determine lime requirement of soils

Many authorities are of the belief that there has been no satisfactory laboratory method developed thus far that can with certainty be substituted for the field-trial method for the determination of the lime requirements of soils. There is, however, a sound theoretical basis for the determination of the lime requirement by the present method.

It has frequently been assumed that when $CaCO_3$ is added to an acid soil the chief chemical reaction is with the so-called "replaceable" hydrogen ions of the exchange complex, i.e., the hydrogen ions replaceable by the base of a neutral salt. From the viewpoint presented it would not be logical to expect any definite relationship between the replaceable hydrogens and the degree of unsaturation of the exchange complex. Consequently, the determination of the replaceable hydrogen would not be a measure of the lime requirements of soils. The neutralization of replaceable H is only one of the factors involved in liming. Nor would it be in accordance with the concepts presented, to use as a basis for the lime requirement a quantity of $CaCO_3$ corresponding to the segment OB, for—because of the presence of $Ca(HCO_3)_2$ in the soil solution (see discussion of the segment OB)—this segment could never become nil even in soils in which large amounts of $CaCO_3$ has persisted for many years. The

complete saturation of the segment OB can take place only in the presence of a slight excess of the hydroxide. It follows that the amount of $CaCO_3$ necessary for neutralization cannot be determined by shaking the soil with an excess of precipitated $CaCO_3$ and then determining the decrease in the segment OB. The saturation of the soil is very slow as a result of the low solubility of $CaCO_3$ even in the presence of small quantities of CO_2.

Clarens (8) has pointed out that if a soluble salt of calcium such as $CaSO_4$ or $CaCl_2$ be added also with the $CaCO_3$, the segment OB is markedly reduced and the reduction is independent within certain limits of the concentration (0.1 to 0.05 N) of the solution used. The reduction in the amount of OB then gives the quantity of $CaCO_3$ necessary for the effective saturation of the soil considered.

The experiments are conducted in the same manner as previously described. Five 1-gm. portions of soil are shaken up with 0.1 gm. of precipitated $CaCO_3$ and 20 cc. of a saturated solution of $CaSO_4$. The solutions are filtered with suction, and the soil is transferred to test tubes. To the first are added 19 cc. of water and 1 cc. 0.1 N $Ba(OH)_2$, to the second 18 cc. water and 2 cc. 0.1 N $Ba(OH)_2$, etc. The tubes are allowed to stand 18 hours, then shaken continuously for 6 hours, and then titrated in the usual manner against 0.1 N HCl and the new segment OB is determined. The "original" soil treated in this way gave OB = 1.10 as against 1.62 before treatment. Therefore, the saturation of the segment due to $CaCO_3 + CaSO_4$ is $1.62 - 1.10 = 0.52$ cc.

If the $CaCO_3$ is omitted and 20 cc. of a saturated solution of $CaSO_4$ only is used, the new segment OB is slightly greater than in the presence of $CaCO_3$ (0.59 as against 0.52). This shows that the $CaCO_3$ has caused a progression of 0 toward A of only 0.07. The conclusion must, therefore, be drawn that the saturation of the soil was due almost completely to the soluble salt of Ca, even when $CaCO_3$ is also present with the soluble salt (8).

This would explain why some investigators have found that when either $Ca(OH)_2$ or $CaCO_3$ is incorporated in one layer of soil (32) or applied as a top-dressing (16) the neutralizing effect is almost nil on the adjacent layers.

Application to determine the existence of different states of availability of the replaceable potassium

Of the many methods which have been proposed to determine the availability of soil potassium the determination of the replaceable form is the most rational and is the one generally favored. But it has already been shown (19) that broad generalizations based on the method are not possible. An additional limitation is imposed on the concept of the identification of the exchangeable potassium with the available potassium if the exchangeable potassium can possess different degrees of availability according to its position in the clay nucleus (7).

Reasoning *a priori* would lead one to expect that an exchangeable base such as potassium would not occupy the same position in the crystal lattice of the

clay nucleus; and within the past few years experimental evidence has been brought to support this premise (4, 22, 23). It would follow, therefore, that the replaceable potassium should exist in different degrees of availability to the plant. If this is the case then the processes of mobilization in which the soil potassium is rendered available would be discontinuous and would be reflected by broken solubility curves.

The "original" surface soil has been investigated from this point of view in the following manner: 200-gm. portions of the "original" air-dry soil contained in 1000-cc. shaking flasks were treated with increasing amounts of 0.1 *N* HCl. To the first flask were added 975 cc. of distilled water and 25 cc. 0.1 *N* HCl; to the second, 950 cc. water and 50 cc. 0.1 *N* HCl; to the third, 925 cc. of water and 75 cc. 0.1 *N* HCl; and to the fourth, 900 cc. of water and 100 cc. 0.1 *N* HCl. The soils were then left in contact for 18 hours and then shaken continuously for 6 hours. Potassium was determined in an aliquot.

As shown in table 4, the replaceable bases of this "original" soil are equivalent to 0.50 cc. 0.1 *N* HCl per gram of soil. The amounts of HCl added to the

TABLE 5

Quantities of potassium liberated from the original soil by treatment with dilute solutions of HCl sufficient to satisfy 25, 50, 75, and 100 per cent, respectively, of the replaceable bases

0.1 *N* HCl ADDED	AMOUNT K_2O DISSOLVED	0.1 *N* HCl EQUIVALENT
cc.	*p.p.m.**	*cc.*
25	40	1 69
50	42	1.79
75	95	2.00
100	87	1.89

* Dry soil.

flasks in this experiment accordingly were equivalent to one-fourth, one-half, three-fourths, and the whole of the replaceable bases, respectively. The results are given in table 5.

These results indicate that, within the range of concentration of HCl used, the solubility curve for the replaceable potassium is a discontinuous one.

Application to determine the changes produced by cultivation and fertilizer dressings

Although the aforementioned problems comprise the principal uses to which the method is adapted, it is of interest to ascertain what changes the method reveals in the properties of the initial condition of the soil at the end of the experimental period of 7 years. After introduction into the cylinders, both check and NPK soils were green manured each year with rye. The check soil received no mineral salts. The NPK soil received, during the whole experimental period of 7 years, total C. P. salt additions as follows: 465.5 gm. N, 1052.8 gm. P_2O_5, 1898 gm. K_2O—equivalent respectively to 1591 pounds N,

3598 pounds P_2O_5, 1898 pounds K_2O per acre 7 inches of 2,000,000 pounds. The precipitation during the whole experimental period was 260 inches, of which 96 inches fell during the experimental period in which the soil received mineral salts. All three soils are low in humus material. In spite of the green manuring, the humus material in the check and NPK soils was still relatively low, viz., <1.5 per cent.

The summarized results of the analytical data for the "original" soil and also for the check and NPK soils are given in table 6.

The divergences in the results of the check soil from the "original" soil are an indication of the joint effects of the growth of the trees and of the humus material from the green manures together with the climatic factors. The degree of unsaturation as represented by the segment OB has increased the equivalent of 0.06 cc. 0.1 N $Ba(OH)_2$ per gram of soil. The replaceable bases

TABLE 6

Comparative data for the original soil, the check, and NPK cylinders

SOIL	SEGMENT OA	SEGMENT OA′	SEGMENT AA′	SEGMENT OB	TAN < CAc	TAN < dBD
Original	0.76	0.50	0.26	1.62	0 84	0.82
Check	0.80	0.45	0.35	1.68	0 72	0.79
NPK	0.85	0 43	0 42	1 73	0 60	0 73

OA = Acid functions saturated by bases including free Fe and Al expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

OA′ = Acid functions saturated by the so-called "replaceable bases" expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

AA′ or OA − OA′ = "Free" $Al(OH)_3$ and $Fe(OH)_3$ expressed in terms of cubic centimeters of 0.1 N HCl per gram of soil.

OB = Unsaturated acidic functions the Ba salts of which are not hydrolyzed by water free from CO_2 in the presence of a slight excess of base. Expressed in terms of cubic centimeters of 0.1 N $Ba(OH)_2$ per gram of soil.

in the check soil have been reduced by the equivalent of 0.05 cc. 0.1 N HCl per gram of soil, whereas the "free" aluminum and iron have been increased by 0.09 cc. 0.1 N HCl per gram of soil. A decrease in the tangent of the angle CAc from 0.84 to 0.72 has occurred, which indicates an increment from a saturation of 16 per cent to a saturation of 28 per cent in the hydroxyl groups marked (A). The most rational explanation would appear to be that this increase is the result of the "deactivating" properties of humic acids (30).

The values for the NPK soil differ more widely from the corresponding values for the "original" soil than do those of the check soil. Particularly to be noted is the *increase* of the segment OB equivalent to 0.11 cc. 0.1 N $Ba(OH)_2$ per gram of soil; the *decrease* equivalent to 0.07 cc. 0.1 N HCl per gram of soil in the replaceable bases; and the relatively large increase equivalent to 0.16 cc. 0.1 N HCl per gram of soil in the "free" aluminum and iron hydroxides. The decrease in the tangent of the angle CAc is equivalent to 0.24, indicating

an increase of 24 per cent in the saturation of the hydroxyl groups marked (A) and representing the net effect of the deactivating properties of humic acids and the phosphate additions. In laboratory studies, Clarens (5) has reached the conclusion that salts of K, Na, NH_4, and Ca do not sensibly modify the unsaturated acid functions. The present experiment indicates that the resultant effect of the $NaNO_3$, K_2SO_4, and $CaH_4(PO_4)_2$ added as fertilizers upon the segments OA, AA′, and OB is similar in magnitude and direction to that produced by the green manuring alone. Moreover, it is a noteworthy fact that the salt additions have not caused any increase in the segment OA′ but, on the contrary, have diminished it slightly. It is the segment AA′ that has increased evidently at the expense of the so-called "replaceable bases."

SUMMARY

By the treatment of a soil with increasing amounts of solutions of hydrochloric acid and also of barium hydroxide in low concentrations, differences in specific properties of the three types of hydroxyl groups of clays may be used as a means for the rapid characterization of a soil with respect to the degree of unsaturation, the total replaceable bases, the "free" aluminum and iron hydroxides, the phosphoric acid required to satisfy the Al and Fe, the lime requirement, the availability of the different states of the replaceable potassium, and the changes produced by cultivation and fertilizer treatment.

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THE APPLICABILITY OF ALKALINE PERMANGANATE FOR OXIDATION OF ORGANIC MATTER IN SOILS FOR MECHANICAL ANALYSIS

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The successive developments and the present position of mechanical analysis of soils have been traced elsewhere (3). The International-A method was slightly modified in Versailles in 1934 according to the recommendation of Robinson (5), who advocates the use of 4 cc. *N* sodium hydroxide to 10 gm. soil and 500 cc. water instead of ammonia, which was previously prescribed for dispersion of soils. A similar conclusion was reached earlier by Chakraborty and Sen (2) while analyzing Indian lateritic soils. These soils refused to disperse fully unless they were shaken and converted into a suspension having a pH of about 10.5. For this purpose about 6–8 cc. *N* sodium hydroxide was required for 20 gm. of soil in 2 liters of water. Although Robinson's recommendations for the pretreatment of different types of soils for mechanical analysis appear to be superior to other proposals made so far, they are not altogether free from limitations. Hydrogen peroxide, which has been recommended for removal of organic matter, is a very costly and somewhat unstable reagent in tropical countries like India and it is catalytically decomposed by many soils. It has also been mentioned by Troell (6) that a fen soil with 37.7 per cent loss on ignition required more than 400 cc. of hydrogen peroxide used over 2 days for removal of organic matter. Similar difficulties have been encountered in the case of Indian soils having high loss on ignition. It is also to be remembered that any method which prescribes, as far as possible, the same mode of treatment for different types of soils in the course of mechanical analysis will be valuable because of the comparability of the results obtained by the particular method.

A new method (2) of dispersing soils involving the use of alkaline permanganate for oxidation of organic matter has been found successful in the case of Indian lateritic soils as shown by agreement of the results with those obtained by the International-A method. This method, which has been termed the "alkaline permanganate method," consists in oxidizing the soil organic matter

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TABLE 1

Oven-dry clay and clay + silt in air-dry soil by different methods

SOIL NO.	LAB. NO.	LOCALITY	INTERNATIONAL-A METHOD H_2O_2-HCl-NaOH (1 PER CENT SUSPENSION OF 20 GM. SOIL SHAKEN FOR 24 HOURS WITH 8 CC. N NaOH)			ALKALINE PERMANGANATE METHOD (1 PER CENT SUSPENSION OF 20 GM. SOIL SHAKEN FOR 6 HOURS WITH 8 CC. N NaOH)		
			Clay	Clay + silt	Loss on solution	Clay	Clay + silt	Loss on solution
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	105	Bengal Dacca Bikrampur	21.2	62.3	1.5	21.2	62.6	1.8
		*Bengal soils**						
2	107	Rajshahi Farm	23.1	58.2	0.8	23.6	56.4	1.0
3	108	Berhampur Farm	19.8	47.1	0.9	20.1	47.5	1.0
4	109	Barisal	23.1	87.2	1.4	23.5	88.0	1.8
5	110	Jessore	17.2	38.1	0.9	18.6	40.2	1 0
6	111	Jalpaiguri Maynaguri Farm	9.1	44.8	1.2	9.3	45 5	1.4
7	112	Siliguri	5.5	14.0	1.5	5.5	13.6	1.4
8	113	Midnapore	20.5	47.8	0.7	20.5	47.3	0.6
		Bengal forest soils†						
9	137	....	6.6	15.6	1.6	6.9	15.7	1.8
10	140	....	9.1	18.5	2.2	10.3	19.2	2.1
11	143	....	14.0	29.6	1.5	12.7	28 7	1 5
		Tobacco and other soils‡						
12	120	Coorg Nulkund Estate Soil I	45.7	62.6	1.4	44.5	60.0	2.6
13	121	Assam Jorhat	9.0	23.8	0.7	10.5	23.4	0.7

* Sent by the agricultural chemist, Bengal.

† Sent by the divisional forest officer, Darjeeling, Bengal.

‡ Sent by Rao Bahadur Biswanath, Imperial agricultural chemist.

TABLE 1—*Concluded*

SOIL NO.	LAB. NO.	LOCALITY	INTERNATIONAL—A METHOD H_2O_2-HCl-NaOH (1 PER CENT SUSPENSION OF 20 GM. SOIL SHAKEN FOR 24 HOURS WITH 8 cc. *N* NaOH)			ALKALINE PERMANGANATE METHOD (1 PER CENT SUSPENSION OF 20 GM. SOIL SHAKEN FOR 6 HOURS WITH 8 cc. *N* NaOH)		
			Clay	Clay + silt	Loss on solution	Clay	Clay + silt	Loss on solution
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
		Tobacco and other soils‡—Concluded						
14	122	Madras Guntur Tobacco soil	43.8	56.6	0.9	44.0	57.2	1.6
15	123	Bombay Gujarat, Sursa Tobacco soil	14.9	24.5	0.8	15.6	25.6	1.1
		Black cotton soils§						
16	114	Mahalung (Nira, right bank canal)	56 2	67.9	1 8	57.1	67.9	2 0
17	115	Durgapur (Provara, left bank canal)	52.9	71.9	1.7	54.3	71.4	2.4
18	116	Lohagaon (Provara, left bank canal)	45.7	59.7	1.8	47.6	59.9	3.0
19	117	Nimbut (Nira, left bank canal)	57.1	71.9	1.6	57.2	72.1	2.2
20	118	Velapur (Godavari, right bank canal)	50 3	68 6	1.6	48.9	66.8	2.0
21	119	Sonewadi (Godavari, right bank canal)	49.1	64 4	1.7	49 8	64 2	2.5

§ Sent by Dr. J. K. Basu, soil physicist, Padegaon, Bombay. Soils 16, 19, and 20 contain, respectively, 11.6, 9.6, and 14.3 per cent $CaCO_3$.

with potassium permanganate in the presence of a little sodium hydroxide. Binding materials, such as calcium carbonate, in soil are removed by hydrochloric acid, and the manganese dioxide precipitated in the process of oxidation is removed by sodium bisulfite in the presence of dilute hydrochloric acid.

The soil is filtered and washed with sodium acetate solution, just barely acidified with hydrochloric acid, until free from manganese and sulfate. The excess of sodium acetate in the soil is washed off with water. The soil is then shaken with sodium hydroxide, made up to a definite volume, and subjected to pipette sampling. In the actual experiment, 20-cc. samples were removed from the 10-cm. depth of the suspension at different times depending on temperature as calculated by Puri and Amin (4) on the basis of Stoke's law.

In this paper the applicability of the alkaline permanganate method has been tested on various types of Indian soils and it has been found that the results of analysis obtained by this method are thoroughly reproducible and comparable with those obtained by the International-A method.

EXPERIMENTAL RESULTS AND DISCUSSION

From table 1 it will appear that the results for clay and clay silt obtained by the International-A method and by the alkaline permanganate method agree

TABLE 2

Oven-dry clay and clay + silt in air-dry gypseous soils by different methods*

SOIL NO.	LAB. NO.	LOCALITY AND DESCRIPTION	$CaCO_3$	$CaSO_4$	INTERNATIONAL-A METHOD (WITH ROBINSON'S MODIFICATION FOR SOILS CONTAINING GYPSUM)		ALKALINE PERMANGANATE METHOD		
					Clay	Clay + silt	Clay	Clay + silt	Loss on solution
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
22	158	Koil patti, 2–8 ft.	8 6	6 8	49 4	57 8	49 1	57 8	2 0
23	159	Guntur, 6–9 ft.	4.1	1 6	57.5	71 5	58 9	73 2	1 9
24	160	Bellary, 2–6 feet.	13 5	2 0	50 1	63 5	49.7	64 1	1 5

*Soils sent by the Government agricultural chemist, Coimbatore, Madras.

very satisfactorily in all the soils. Percentage loss on solution is almost the same by the two methods.

Soils containing calcium carbonate and gypsum

It will be highly interesting to see whether this particular method may be applied without modification in the case of gypseous soils. Robinson (5) has suggested the following procedures for obviating difficulties of dispersion with gypseous soils:

(*a*) Removing coarse gypsum by passing through a 70-mesh sieve after peroxidizing and before acid treatment.
(*b*) Using a stronger acid.
(*c*) Washing with 10 per cent ammonium acetate.

It is to be noted that although the modification (*a*) suggested by Robinson must be included in the alkaline permanganate method after removal of

organic matter in the case of gypseous soils, the other two modifications, (*b*) and (*c*), are unnecessary, for most of the gypsum present in the soil will be transformed into calcium carbonate as a result of reaction between gypsum and alkaline carbonate produced during oxidation of organic matter by alkaline permanganate. It must be stressed, however, that in the case of gypseous soils 15 cc. of *N* sodium hydroxide, in addition to the alkali required with the permanganate for oxidation of organic matter, should be added gradually for each gram of calcium sulfate contained in the soil.

The results for clay and clay silt shown in table 2 agree very closely for the two methods. The percentage loss on solution is noticeably low. These soils were passed through a 70-mesh sieve after oxidation with alkaline permanganate and before addition of hydrochloric acid to remove coarse gypsum.

TABLE 3

Clay and clay + silt in air-dry soil by different methods

SOIL NO.	LAB. NO.	LOCALITY AND DESCRIPTION	LOSS ON IGNITION	INTERNATIONAL-A METHOD			ALKALINE PERMANGANATE METHOD		
				Clay		Loss on solution	Clay		Loss on solution
				Oven-dried	Ignited		Oven-dried	Ignited	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
25	06	Assam peaty Bheel soil sent by Derby Tea Co.	56 0	22 6	15 2	3 2	25 0	17.6	3.5
26	02	Assam peaty soil sent by Sephinjuri Bheel Tea Co. Ltd.	34 5	35.6	29.1	2 8	37.1	30 9	3.8
				Clay + silt			Clay + silt		
25	..	. . . . . .	.	46.1	35.2	...	47.1	35 8	
26	..	. ...	....	62.0	54 5	...	61 1	54 5	...

Soils containing large quantities of organic matter

It was necessary to determine how the soils containing large quantities of organic matter respond to the alkaline permanganate method. For this purpose two soils from Assam having loss on ignition as high as 56 and 36 per cent have been subjected to analysis by both the methods. It has been found that a very large volume of hydrogen peroxide used over about 2 days is required for changing the dark humic color of the soils. Even then the removal of organic matter might not have been so perfect as with the alkaline permanganate, which required about 3 hours to complete oxidation. The results of analysis are given in table 3.

Table 3 shows that although the percentages of clay silt, both oven-dried and ignited, are almost the same by the two methods, the results for clay, both oven-dried and ignited, by the alkaline permanganate method are distinctly

higher than those by the International-A method. Soil 25, i.e., the soil with higher loss on ignition, shows greater difference in clay figures than soil 26. This at once suggests that in these soils dispersion by the alkaline permanganate method has been more complete than by the International-A method. This may be cited as an additional reason for adopting the permanganate method for mechanical analysis of soils. Loss on solution of these soils, as expected, was found to be a little higher than that of other soils.

SUMMARY AND CONCLUSIONS

A method of mechanical analysis using alkaline permanganate for oxidation of organic matter has been found to yield comparable results with the International-A method in the case of various Indian soils, e.g., ordinary arable, forest, gypseous, peat, and lateritic (1) soils. Thus the method appears to be a general method for mechanical analysis of soils. This is particularly suitable for soils rich in organic matter and is recommended for use in tropics where hydrogen peroxide is not very stable.

Alkaline permanganate requires only a short time for oxidation of organic matter.

Soils containing gypsum should be passed through a 70-mesh sieve to remove coarse gypsum after oxidation with alkaline permanganate and before addition of hydrochloric acid.

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DISPERSION OF SOIL FOR MECHANICAL ANALYSIS BY SODIUM CARBONATE OR SODIUM OXALATE TREATMENT

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An examination of existing methods of dispersion, of which many excellent reviews can be found in the literature, reveals the fact that all of them ultimately aim at producing a sodium-saturated soil. In the removal of other exchangeable bases, notably Ca and Mg, whether by HCl treatment (7), NaCl leaching (4), boiling with ammonium carbonate (5), or treatment with sodium carbonate (2) or sodium oxalate (3), the underlying principle is the same, i.e., replacement by Na. Humus if present introduces complications and requires treatment with H_2O_2, but such soils can be easily given special treatment before sodium-saturated soil is produced.

In a previous publication the writer has shown that exchangeable Ca in soils can be completely precipitated as carbonate or oxalate by shaking with a soluble carbonate or oxalate (6). In other words we can convert a Ca-saturated soil into a Na soil by treatment with sodium carbonate or oxalate in quantities equivalent to the exchangeable Ca in it. If we knew the exchangeable Ca and unsaturated acidoid in a soil, it would be a simple matter to add Na_2CO_3 or Na oxalate equivalent to the exchangeable Ca, and NaOH equivalent to the unsaturated acidoid; a sodium-saturated soil would result, and no extraneous chemical would be in excess. The soil in that state will show maximum dispersion.

The extra work involved in the preliminary determination of exchangeable Ca and free acidoid is more than compensated by the fact that the results obtained are valuable in themselves and should prove useful data for any soil under examination. Besides, this determination entails only simple titrations for which the actual working time is much less than that required for most of the preliminary treatments advocated from time to time.

DETAILED DESCRIPTION OF THE PROPOSED METHOD

Ten grams of soil is left with 200 cc. of 0.2 N K_2CO_3 in N KCl for 1 to 2 hours with occasional shaking. The suspension is then filtered and an aliquot titrated for total alkalinity by adding excess of standard acid, boiling, and back titrating with standard alkali. It can be titrated directly, as well, with methyl red as indicator. The total decrease in acidity is equivalent to the exchangeable Ca+ free acidoid. Another aliquot of the filtrate is titrated for bicarbonates by adding known amounts of standard NaOH and $BaCl_2$ and then

titrating the residual NaOH with standard oxalic acid using thymolphthalein as indicator. Phenolphthalein can be used, but the former gives a sharper end point. The total bicarbonates are equivalent to the free acidoid. It is understood, of course, that the original solution contains no bicarbonate; if it does, this quantity is subtracted from the final value. Total decrease in acidity minus the bicarbonates give exchangeable Ca and sodium carbonate or sodium oxalate equivalent to exchangeable Ca, and NaOH equivalent to free acidoid is added to 10–20 gm. of soil suspended in about 200 cc. of water and shaken over night. Two hours' shaking suffices for most soils, but overnight shaking will ensure maximum dispersion in all types. After being shaken the soil is ready to be made up to the desired volume for the pipette method of mechanical analysis.

ALTERNATE METHOD

An alternate method is based on the potassium oxalate method of estimating exchangeable Ca in soils (6). Ten grams of soil is shaken, by hand, in a stoppered bottle for about half an hour with 100 cc. of the following solution and cooled below 15°C.: *N* with respect to potassium acetate, 0.015 *N* with respect to potassium carbonate, 0.1 *N* with respect to potassium oxalate. The suspension, after being shaken, is filtered and an aliquot of the filtrate titrated with standard permanganate. The total decrease in the concentration of oxalate ion is equivalent to the exchangeable Ca in the given weight of the soil. Sodium carbonate or oxalate equivalent to exchangeable Ca is then added to the soil suspended in about 100 cc. of water. The suspension is then brought to pH 10.5 by adding 0.5 *N* NaOH in 1-cc. lots, shaken vigorously, and tested with thymolphthalein as an external indicator until an intense blue color is produced on a drop of the indicator. It is best to start about a dozen or more soils at a time so that sufficient time can be allowed between incremental additions of alkali. The soil suspension is then shaken overnight and made up to the desired volume for pipette analysis.

It might be pointed out that it is not essential to add exact quantities of oxalate-carbonate or hydroxide equivalent to the exchangeable Ca or acidoid; these quantities can be varied within certain limits without affecting the results. This fact leads to a further simplification of technique when dealing with soils of a similar type from a restricted area. The highest amount of exchangeable Ca and acidoid can be ascertained by examining a number of soils, adding a corresponding amount of carbonate—oxalate and hydroxide, and shaking the suspension overnight.

As regards the relative merits of sodium carbonate or oxalate, no opinion can be expressed; both give similar results, and the choice must be left to the individual worker.

EXPERIMENTAL

The sodium carbonate-oxalate method was compared with the author's NaCl-NaOH method, with the $(NH_4)_2CO_3$ method, and in the case of humus soils

TABLE 1

Clay content of soils by various methods of dispersion

SOIL NUMBER	LOCALITY	CLAY CONTENT			
		NaCl method	$(NH_4)_2CO_3$ method	Na_2CO_3 method	$Na_2C_2O_4$ method
		per cent	*per cent*	*per cent*	*per cent*
	Ordinary agricultural soils				
1	Pusa (Bihar)	11.3	13.7	11.9	9.8
4	Ranala I (Punjab)	15.2	18.1	14.3	13.2
5	Ranala II (Punjab)	12.3	14.8	12.4	11.7
8	Coimbator I (Madras)	25.2	25.7	24.9	26.1
10	Coimbator II (Madras)	35.6	37.0	36.7	37.7
11	Coimbator III (Madras)	32.8	31.0	33.2	33.3
17	Kalyanpur (U. P.)	11.9	17.5	10.7	10.0
19	Mandalay (Burma)	42.4	44.2	42.5	41.3
21	Kaing (Burma)	13.5	16.4	11.2	12.1
23	Myetha (Burma)	11.3	11.8	10.2	9.8
26	Hebbal (Mysore)	22.6	20.5	17.2	21.3
28	Nagpur (C. P.)	44.6	44.6	47.7	40.9
31	S. Travancore	22.8	28.1	22.3	22.6
34	Gurdaspur (Punjab)	11.3	15.5	10.1	11.6
35	Gurgaon (Punjab)	18.3	18.3	14.7	16.6
36	Hansi (Punjab)	11.7	14.4	10.4	10.3
43	Churland (Bihar)	19.7	24.7	20.3	20.8
44	Highland (Bihar)	8.4	13.2	8.3	9.7
48	Montgomery I (Punjab)	19.8	24.8	18.6	20.2
50	Tarnab I (N. W. F. P.)	17.7	19.6	17.2	17.8
51	Tarnab II (N. W. F. P.)	12.9	13.9	13.3	13.0
52	Sabour (Bihar)	11.3	10.0	9.2	8.4
54	Montgomery II (Punjab)	3.2	6.2	4.2	4.2
55	Sakrand I (Sindh)	11.0	14.9	12.1	12.2
56	Sakrand II (Sindh)	13.1	17.5	18.2	15.8
59	Raghopur (U. P.)	10.9	12.2	9.3	9.0
	Black cotton soils				
2	Akola (Bombay)	59.3	62.8	59.0	60.3
3	Dharwar (Bombay)	62.2	63.8	63.9	65.0
13	Nagpur (C. P.)	58.9	60.4	60.5	57.7
27	Babhur (Mysore)	53.2	59.1	54.1	57.9
29	Jabbalpur (C. P.)	63.0	57.4	64.0	64.7
30	Hoshangabad (C. P.)	54.1	55.1	54.6	56.6
41	Poona (Bombay)	53.4	61.6	57.0	57.7
42	Khandesh (Bombay)	53.4	58.9	58.8	61.2
46	Baroda (Bombay)	56.6	59.0	58.0	60.2

TABLE 1—*Concluded*

SOIL NUMBER	LOCALITY	CLAY CONTENT			
		NaCl method	$(NH_4)_2CO_3$ method	Na_2CO_3 method	$Na_2C_2O_4$ method
		per cent	*per cent*	*per cent*	*per cent*
	Lateritic and ferruginous soils				
6	Dacca (Bengal)	28.4	31.6	30.4	29.3
9	Malabar (Madras)	21.6	22.8	22 6	24.3
12	Tocklai (Assam)	3.8	14 8	9 8	9.1
14	Estate Soil (Madras)	11 1	31 5	34.7	35.6
15	Shillong (Assam)	19.4	27.6	25.6	29.5
18	Mandalay (Burma)	19.9	25.2	19 8	20.1
20	Lower Burma	6.5	15.1	10 6	8 2
22	Pyrinana Soil (Burma)	15 2	17.8	14 0	13.3
25	Bhur Soil (U. P.)	4 0	5.8	4 2	3 8
37	Rangpur (Bengal)	4 2	15 3	6 6	7 9
40	Umareth (Bombay)	13 1	17.0	12.3	13.9
45	Bihar	10.7	14 0	10.5	8 6
49	Madhupur (Bihar)	27.3	32.6	26 3	27.1

with the International method. Altogether, 56 soils, comprising practically all the types found in India, were examined. For convenience of reference, they are grouped as follows: ordinary agricultural soils, black cotton soils, lateritic and ferruginous soils, humus soils.

The results are given in tables 1 and 2.

DISCUSSION OF RESULTS

If the highest clay percentage obtained by any method be the criterion, then the $(NH_4)CO_3$ method appears to be the most efficient. It was found, however, that for some soils even H_2O_2 treatment failed to give as much dispersion aş that obtained with $(NH_4)_2CO_3$. This raised the doubt that in certain soils $(NH_4)_2CO_3$ might bring into suspension material other than clay, and the high alkalinity of the medium would lend support to this view.

The sodium carbonate-oxalate method produced maximum disperson in all soils except the humus soils. Even those soils that responded poorly to the NaCl-NaOH method gave very good results with this method.

The sodium carbonate-oxalate method is of particular interest, for apart from its extreme simplicity it has a theoretical background which places the whole question of clay dispersion on a sounder footing. It affords us for the first time the true explanation of the empirically discovered superiority of sodium carbonate and sodium oxalate for dispersing soils and the cause of their failure when not used in the right quantities. The work of Chakraborty and Sen (1), who showed that laterite soils require only shaking with NaOH to give maximum dispersion, is also brought into line with the logical deductions of the principle involved in the present method. Laterite soils are highly unsaturated

soils which contain very little exchangeable Ca. Such soils, therefore, require only neutralization of the acidoid with NaOH to attain maximum dispersion.

Humus soils are the only type which fails to respond to the sodium carbonate-oxalate method (table 2). The cause of this failure seems to lie in the mechanical difficulty of breaking up the clay-humus aggregate. Clay in highly humus soils is more or less enveloped in a humus layer which must be broken down mechanically to enable the clay particles to go into suspension. The action of H_2O_2 in the oxidation of humus, as far as the dispersion of clay is concerned, is still not well understood. No doubt some oxidation takes place, but the loss on ignition of the oven-dry clay determined after the H_2O_2 treatment indicates that the portion actually destroyed is immaterial.

The only modification in the sodium carbonate-oxalate method to suit humus soils is as follows: After the addition of the right quantities of sodium carbonate or oxalate and hydroxide, the soil suspended in 100–200 cc. of water is boiled

TABLE 2

Clay content of humus soils

SOIL NUMBER	LOSS ON IGNITION	CLAY CONTENT							
		Na_2CO_3 method		$Na_2C_2O_4$ method		H_2O_2 method		$(NH_4)_2CO_3$ method	
		Oven dry	Ignited	Oven dry	Ignited	Oven dry	Ignited	Oven dry	Ignited
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
75	50.1	24 4	9 2	18.2	5.8	37 2	15 0	36 7	15.5
76	35.7	14 2	5 7	17 3	7 1	29 7	18 3	29.4	15 5
78	13.1	15 1	9 6	17 5	10 4	25 7	19.9	30 1	16.0
79	12.3	14 2	8 7	16 1	9 8	26 0	20 0	27 5	19.6
81	35 9	18 0	6 8	20.2	7 5	31 2	15.3	28 6	20.2
84	32 2	11 5	5 3	13 6	5 8	28 5	15 7	32 0	17 8
86	29.9	12 1	5 2	14 0	6 2	23 8	16 4	34.2	15 3
87	28.7	12 6	5 9	13 8	6 2	27 3	13 9	27 9	19 7

for 1 to 2 hours, with small quantities of H_2O_2 or $(NH_4)_2CO_3$ added at intervals to keep up a brisk boiling. The disruption of the clay humus complex is effected by bubbles of oxygen in the one case and by CO_2 in the other. Frothing can be prevented by the addition of a little mineral oil.

As will appear from the loss on ignition of humus soils given in table 2, soils were specially selected with very high humus content to put the proposed method to an extremely rigid test. Ordinarily, soils with such a high humus content, when shaken with KCl-K_2CO_3 for the preliminary estimation of exchangeable Ca and free acidoid, yield a colored filtrate. However, with a certain amount of dilution and back titration technique and the use of brom thymol blue as indicator there is generally no difficulty in locating the end points with reasonable accuracy. As has been pointed out, it is not necessary to add exact amounts of reagents for final dispersion.

Clay in humus soils is generally recorded on the ignited weight basis. This practice is open to objection as the loss on ignition is not a fixed constant but

must vary with the manner in which ignition is carried out. This will produce lack of uniformity of results in the hands of different workers. Humus itself would leave on ignition a mineral residue that could not be classed as clay by any stretch of imagination. The practice of taking the ignited weight evidently started with the object of rigidly excluding everything that was not clay; but it is bound to lead to a distorted vision of the true state of affairs. For all practical purposes humus is as good a clay as clay can be. It shows base exchange, moisture absorption, and other related properties like clay and therefore must be classed as clay. Besides, its manner of dispersion is not fundamentally different from that of clay, and the results obtained are just as reproducible. From the practical point of view, also, humus plays as important a rôle as clay, and there is no reason why it should not be reckoned as such. In table 2 are recorded both the ignited and the oven-dry weights. It will be seen that results on the oven-dry basis with the H_2O_2 and $(NH_4)_2CO_3$ treatment show a good agreement, indicating that in both cases we are measuring a fundamental constituent of the soil and not something that is produced as the result of a particular treatment.

It is obvious that soils containing excessive amounts of soluble salts or gypsum cannot be dispersed by any single treatment, for the salts will appear as clay even if the soil does not become flocculated. Such soils must be first leached with water to rid them of soluble salts and then shaken with $BaCO_3$ to make the calcium sulfate ineffective (6).

SUMMARY

The proposed method consists in the estimation of exchangeable Ca and free acidoid, sodium carbonate or oxalate equivalent to the former and sodium hydroxide equivalent to the latter being subsequently added and the soil suspension shaken overnight.

To attain maximum dispersion, soils rich in organic matter require boiling with H_2O_2 or ammonium carbonate in addition to the foregoing treatment.

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BEHAVIOR OF POLYVALENT CATIONS IN BASE EXCHANGE[1]

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The theoretical and practical significance of base exchange in soil formation and in plant nutrition has been fully discussed in previous papers (2, 4). The present study aims to ascertain further the rôle played by various cations in the exchange process. Emphasis is laid on carefully standardized and controlled experimental conditions in order to permit quantitative comparisons of the replacing power of the electrolytes investigated.

EXPERIMENTAL TECHNIQUE

In general, the technical procedure employed was the same as that reported in earlier publications (1). Clays extracted from the subsoil of the Putnam silt loam were electrodialyzed, and the H-clays thus formed converted into basic clays by addition of hydroxides, except in the case of La- and Th-clays which were obtained by leaching of NH_4-clay with $La(NO_3)_3$ and $ThCl_4$. In all experiments 7.5 gm. of colloidal Putnam clay (weight expressed as H-clay) containing 4.50 m.e. exchangeable cations was put into a 500-cc. flask, a known amount of electrolyte added, and the container filled to the mark with doubly distilled water. After a reaction time of one week, during which the flasks were kept in a constant temperature room (28 ± 1°C.), the extent of the exchange was determined from analyses of the supernatant liquid.

TABULATION OF DATA

The results obtained are reported in tables 1–5. To economize in space, only the initial electrolyte concentrations and the corresponding exchange values are given. Any desired equilibrium value can be easily computed from these data. The amount of electrolyte added is expressed in terms of symmetry concentrations (S), viz., multiples of the milliequivalents of exchangeable ions in the colloidal system. For the clay concentrations used, 1S corresponds to 4.50 m.e. of electrolyte per 500 cc. volume. The exchange is listed as percentage of adsorbed ions replaced (4.50 m.e. = 100 per cent).

[1] Contribution from the department of soils, Missouri Agricultural Experiment Station, Journal Series No. 475.

[2] The authors are indebted to the National Research Council for a grant-in-aid in 1933. The work was completed in 1934, but publication had to be delayed.

TABLE 1

Ionic exchange with NH_4-clay

(Released NH_4 ions determined)

NATURE OF ELECTROLYTE ADDED	IONIC EXCHANGE VALUES FOR THE FOLLOWING SYMMETRY CONCENTRATIONS			
	½	1	2	4
LiCl	20.56	29.89	44.44	54.67
NaCl	20.56	(35.33)	45.11	54.89
KCl	32.78	51.33	66.78	75.22
RbCl	41.11	62.56	80.22	90.00
CsCl	51.33	68.78	85.33	90 00
HCl	50.44	84.89	90.89	90 89
$MgCl_2$	48.67	65.44	71.29	78 37
$CaCl_2$	45 44	63 56	75.33	80.00
$SrCl_2$	52.22	69 44	77.00	82.67
$BaCl_2$	52.67	71.67	77.00	83 11
Luteo chloride (3 valent) (cobalt determined)	48.8	96.1	102 4	103.8
$ThCl_4$	46.00	80.89	92 22	92 44
Methylene blue	.....	83 33	.. ..	..

TABLE 2

Ionic exchange with Ca-clay

(Released Ca ions determined)

NATURE OF ELECTROLYTE ADDED	IONIC EXCHANGE VALUES FOR THE FOLLOWING SYMMETRY CONCENTRATIONS			
	½	1	2	4
LiCl	9 49	13 08	19.52	27 45
NaCl	9 08	12 74	19.72	30 16
KCl	18 98	28 80	42 29	59 37
NH_4Cl	18 91	29.35	43.99	62 42
RbCl	28 46	43 85	63 03	80 99
CsCl	30 94	50 83	70 07	89.90
HCl	47.57	77 80	87 80	89 19
$MgCl_2$	32 11	47 53	63 32	76 67
$BaCl_2$	35 13	52 96	67.00	80.78
$ThCl_4$	42 90	80 24	95.56	99.35
Methylene blue	..	77.94	.....	.....

TABLE 3

Ionic exchange with K-clay

(Released K ions determined)

NATURE OF ELECTROLYTE ADDED	IONIC EXCHANGE VALUES FOR THE FOLLOWING SYMMETRY CONCENTRATIONS			
	½	1	2	4
HCl	52.36	84.44	86.53	95.43
$CaCl_2$	43.70	60.30	76.42	72.17
$La(NO_3)_3$	49.72	83.48	87.49	86.85
$ThCl_4$	47.85	82.88	90.57	90.89

NATURE OF EQUILIBRIUM

The data in tables 1–5 reveal that the base exchange equilibrium is not always a true one. Different exchange values are obtained for the left hand

TABLE 4

Ionic exchange with H-clay

NATURE OF ELECTROLYTE ADDED	IONIC EXCHANGE VALUES FOR THE FOLLOWING SYMMETRY CONCENTRATIONS			
	½	1	2	4
LiCl	4.5	6.6	9.0	13 0
NaCl	3.5	6.2	9.8	13 5
KCl	8 6	14.5	26 0	34 2
RbCl	19.01	28 20	41 22	62.16
CsCl	25 20	39 73	60 24	82 88
	EXCHANGE DETERMINED BY ANALYSES OF THE DI-VALENT CATION BEFORE AND AFTER EXCHANGE			
$MgCl_2$	10.67	15 78	22 44	..
$CaCl_2$	17 33	26 89	44 00	.. .
$SrCl_2$	30.22		. .	.....
	24 44	34 55	54 67	. ...
$BaCl_2$	16 00	23.78	34 44	.

TABLE 5

Ionic exchange with poly-clays and chlorides

(Outgoing ion determined)

NATURE OF CLAY	NATURE OF ELECTROLYTE ADDED	IONIC EXCHANGE VALUES FOR THE FOLLOWING SYMMETRY CONCENTRATIONS			
		½	1	2	4
Mg-clay	KCl	20 95	31 32	45 28	63.04
Mg-clay	HCl	50 27	79 79	88 77	94 35
Mg-clay	$BaCl_2$	28 73	50 27	71 01	83 58
Sr-clay	KCl	16 40	25 76	39 38	54 22
Sr-clay	HCl	43 31	68 93	78 89	82 15
Ba-clay	KCl	17 61	26 75	39 89	57 32
Ba-clay	HCl	45.99	71 03	83 60	88 36
La-clay	KCl	9 62	13 96	21 34	33 74
Th-clay	KCl	1 85	1 85	3 11	5 56

and the right hand approach of the final state. When, for example, the system

$$\underline{\text{clay}|}\,\text{Ca} + 2\text{KCl} \rightleftharpoons \underline{|\text{clay}|}^{-}_{\text{K}} + \text{CaCl}_2$$

is considered, it is found that at symmetry concentrations 28.8 per cent Ca from Ca-clay is replaced by addition of KCl, whereas 60.2 percent K is released from

K-clay + $CaCl_2$. The sum of the two values is only 89.0 per cent instead of 100 per cent. The discrepancy is particularly noticeable in exchange reactions involving both monovalent and polyvalent cations (e.g., Th-systems). These data confirm the observations of Vanselow (5), who is of the opinion that the phenomenon, which he calls "hysteresis," is associated with the crystal structure of the colloidal particle.

DEFINITION OF ADSORPTION

The term "adsorption" as used in this paper designates a change in ion concentration in the surface without any implication regarding the nature of the forces active in the reaction. If we state that a particular ion has been "adsorbed" we merely indicate that the concentration of that ion in the solid phase has been increased. The adsorbed ions may or may not be exchangeable. If they are exchangeable we assume that the exchange reaction is stoichiometric.

BEHAVIOR OF MONOVALENT CATIONS

The adsorption of monovalent cations by mono- and poly-clays yields typical lyotropic series (fig. 1) which can be described as follows:

$$\mathrm{Li} \lesseqgtr \mathrm{Na} < \mathrm{K} < \mathrm{NH_4} < \mathrm{Rb} < \mathrm{Cs} < \mathrm{H}$$

The peculiar crossing of the Li and Na curves confirms previous observations (2). An important result of the present study is the fact that the lyotropic series of the monovalent ions appears to be a characteristic property of a given colloidal clay system. The relative sequence of the ions within the series remains the same whether the system is an NH_4-clay, a H-clay, or a Ca-clay. The release of surface ions produces a series which is exactly the reverse of the adsorption order. Again, the series is independent of the nature of the cation which brings about the release.

These results lead one to believe that monovalent cations adsorbed on clay surfaces possess certain characteristic properties (e.g., ion hydration, oscillation space, etc.), which are not greatly influenced by the neighboring exchangeable cations.

BEHAVIOR OF POLYVALENT IONS

The polyvalent ions studied comprise di-, tri-, and tetravalent cations, all of rare gas type. Although we analyzed a considerable number of exchange reactions, the data at hand are not numerous enough to permit enunciation of generalized principles because the ions seem to act in an irregular manner.

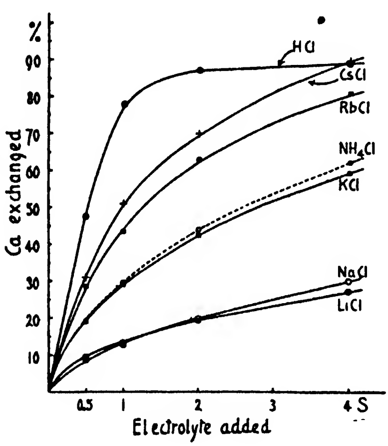
Figure 2 shows exchange isotherms for NH_4-clay systems (compare table 1) whereas figure 6 depicts the results with K-clays (compare table 3). From these data the lyotropic series for polyvalent ions can be ascertained. It is as follows:

$$\mathrm{Mg} \lesseqgtr \mathrm{Ca} < \mathrm{Li} < \mathrm{Ba} < \mathrm{La} < \mathrm{Th}$$

The position of the two lowest members of the series, Mg and Ca, becomes reversed with increasing electrolyte concentration, a feature also observed for the first two ions in the monovalent series (Li, Na). Generally speaking, the curves for the divalent ions assemble themselves into a relatively closely packed bundle of lines, and in this respect the Putnam clay differs significantly from the permutite systems previously investigated (fig. 3). Unlike the monovalent ions the divalent ones do not produce consistent adsorption series, as seen from the following tabulation:

NH_4-clay gives the order Mg $\lesseqgtr$ Ca < Sr < Ba,
H-clay gives the order Mg < Ba < Ca < Sr,
Ca-clay gives the order Mg < Ca < Ba.

In the case of H-clay, Ba has shifted to a lower position, and the Mg- and Ca-curves do not overlap.



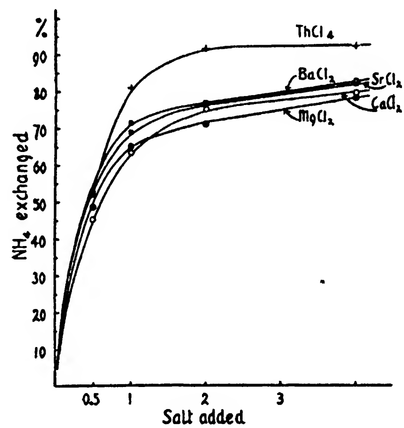


FIG. 1 FIG. 2

FIG. 1. EXCHANGE ADSORPTION OF MONOVALENT CATIONS BY CA-CLAY
FIG. 2. EXCHANGE ADSORPTION OF DIVALENT AND TETRAVALENT CATIONS BY NH_4-CLAY

In the process of release of adsorbed divalent cations the irregularities observed during intake become amplified. A few typical examples may serve as illustrations. If Mg-, Ca-, Sr-, and Ba-clays are treated with KCl or with HCl, the divalent ions come out in the order

Sr < Ba < Ca < Mg,

which differs from the negative adsorption series in that Sr and Ba are not reversed. Exchange of Mg-clay and Ca-clay with $BaCl_2$ produces the relation Mg $\gtreqless$ Ca, according to which the Mg-curve and the Ca-curve cross each other (fig. 4). At low electrolyte concentration Mg appears to be held more tightly than Ca, whereas at higher salt concentration the reverse is true. At present it appears difficult to furnish an explanation of these irregularities.

INFLUENCE OF IONIC SIZE

There are two comparable sets of results which permit one to evaluate the rôle of ionic diameters in base exchange. The two systems are

NH_4-clay + monovalent cations (Li, Na, K, Rb, Cs), and
NH_4-clay + divalent cations (Mg, Ca, Sr, Ba).

In figure 5 the symmetry values have been plotted against the *ionic radii* (crystal lattice radii of Goldschmidt). For both systems an S-shaped curve is the characteristic feature of the base exchange-ionic size relationships. A still more pronounced wave nature is observed for the curves of Ca-clay and H-clay, the data of which are contained in tables 2 and 4.

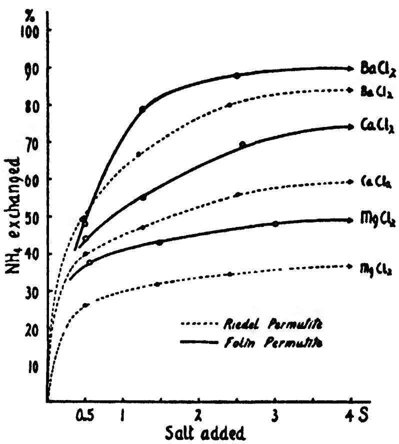


FIG. 3

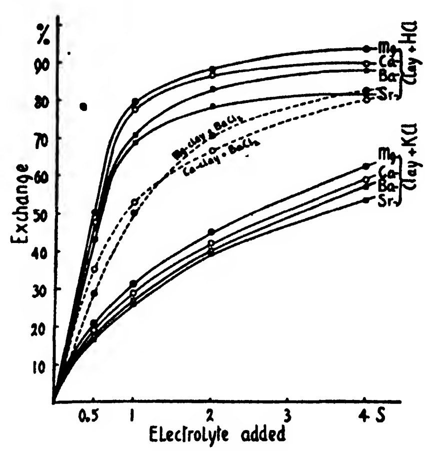


FIG. 4

FIG. 3. ADSORPTION OF DIVALENT IONS BY TWO NH_4-PERMUTITES
Note the wide spread of the curves as compared with NH_4-clay in figure 2

FIG. 4. RELEASE OF DIVALENT IONS (MG-CLAY, CA-CLAY, ETC.) BY MONO- AND DIVALENT CATIONS

With Wiegner, we believe that the hydration of ions offers at present the best explanation of the lyotropic series. Wiegner's original concept, however, needs slight modification in that the hydration of ions assumes different magnitudes in different silicate systems and thus cannot be directly related to hydration values of ions in dilute solutions. In Putnam clays the small ions (Li, Mg) act as if they were partially dehydrated.

Recently, certain Dutch investigators [Verwey (6), Bär (1)] have suggested that the lyotropic series can be explained by polarization of ions, thus eliminating the hydration concept, which, as is admitted, is not satisfactory in all respects. Calculations show, however, that the attraction energy due to ion polarization is only a fraction of the straight Coulombic attraction, and thus unable to reverse the lattice radii series. From the publications at hand it is not quite clear how the aforementioned investigators visualize the

origin of the observed lyotropic series on the basis of polarizability of the exchangeable ions.

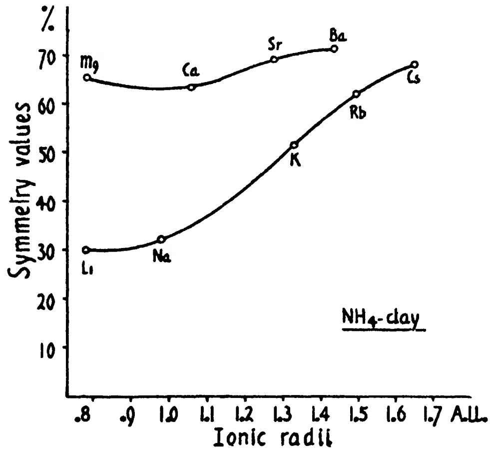


FIG. 5. EXCHANGE INTENSITIES PLOTTED AS A FUNCTION OF THE RADII OF THE ADSORBED IONS

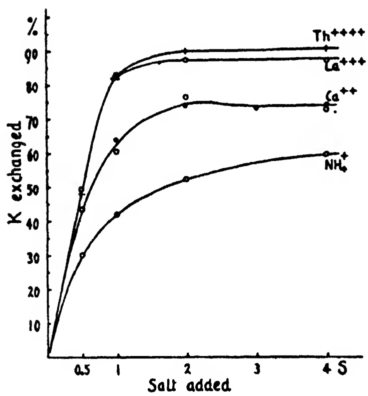


FIG. 6

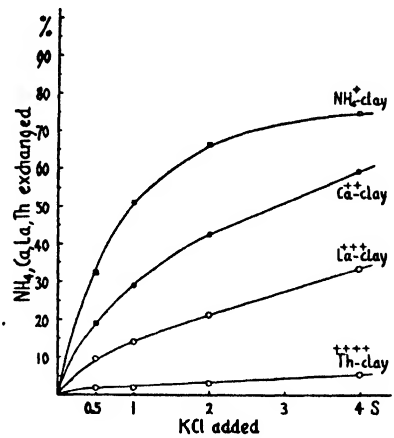


FIG. 7

FIG. 6. EFFECT OF ELECTRIC CHARGE OF IONS ON THE ADSORPTION INTENSITY
K-clay treated with chlorides

FIG. 7. RELEASE OF ADSORBED MONO- AND POLYVALENT CATIONS BY KCl

INFLUENCE OF THE ELECTRIC CHARGE OF IONS OF SIMILAR SIZE

For ions of similar size the adsorption intensities can be correlated with the electric charge. The present study permits the comparison of the following ions, the radii of which are given in parenthesis (Angstrom units):

K^+ (1.33), NH_4^+ (1.43), Ca^{++} (1.02), La^{+++} (1.22), Th^{++++} (1.10)

Potassium and ammonium ions are very similar in size and behave practically alike in the exchange process (*see* fig. 1). Table 3 contains the experimental data for the adsorption of Ca, La, and Th by K-clay, whereas the values for the system K-clay + NH_4Cl are contained in a previous publication (1). Unfortunately these earlier data are not strictly comparable with the present ones. The curves are shown in figure 6; it is evident that the order of ions in adsorption is as follows:

$$NH_4^+ < Ca^{++} < La^{+++} < Th^{++++}$$

The higher the charge of an ion, the better it is adsorbed. The effect of valency on the release can be studied from the following systems:

NH_4-clay + KCl (table 1)
Ca-clay + KCl (table 2)
La-clay + KCl (table 5)
Th-clay + KCl (table 5)

The exchange curves are represented in figure 7, according to which the order of release takes the following form

$$Th^{++++} < La^{+++} < Ca^{++} < NH_4^+$$

It is the reverse of the adsorption series. Monovalent NH_4^+ comes out most easily; Th^{++++}, with four electric charges, is released with great difficulty. All clays are negatively charged (4).

SUMMARY

A study has been made to ascertain the rôle of mono- and polyvalent cations in base exchange reactions with Putnam clay. Although the behavior of the ions is irregular, it appears that the electric charges and the sizes of the ions are two of the major factors which determine the position of an ion in the adsorption and release series.

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THE EFFECT OF PHOSPHATES ON THE CATION EXCHANGE CAPACITY OF CERTAIN SOILS[1]

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The literature on base exchange studies on soils has been voluminous in recent years. The earlier work by men like Kelley (8), Chapman (6), Schollenberger (25), Pierre (18), and Puri (20) was largely concerned with methods of studying replaceable bases. Then followed work on the nature of base exchange material (9, 10, 11), the relation of exchangeable cations to the physical properties of soil (2), and the intricate relation between cation and anion exchange with aluminum, iron, and silicate complexes (3, 12, 15).

In all these studies the phosphates play a very important rôle, and the literature on the subject of the mechanism of phosphate adsorption or retention is growing tremendously. The work of Scarseth (23, 24), Ravikovitch (21), Roszman (22), and Mattson (15) may be cited in this connection.

Recently the authors of this paper have been carrying on base exchange studies on soils that have received various nitrogenous fertilizers, especialy ammonium compounds. In addition to receiving different nitrogen fertilizers, these soils have been receiving three different applications of superphosphate. This fact suggested the possibility of studying the relation between the cation exchange capacity of a soil and the amount of phosphates applied. Sassafras silt loam soil from one of the cylinder experiments at the New Jersey Station, where different nitrogenous materials have been applied annually since 1922, was used in this study. Originally the soil was strongly acid, having a pH of 5.3. This acid condition was corrected by the use of ground limestone to bring the reaction to about 6.6–7.0. At intervals during the 14-year period of this experiment lime has been applied when the pH value of the soil dropped to about pH 6.0 or a little lower. Unfortunately, about 2 years ago the pH value of the soil on section C had dropped to about 5.0. At that time ground limestone was applied to bring the pH to 6.6–7.0. No lime was applied to sections D and E and as a consequence these are becoming more acid, and at the present time are about 0.2 to 0.3 of a pH unit below 6, whereas the pH of the soil in section C is close to pH 6.5. These fluctuations in pH should be taken into consideration when the exchangeable hydrogen and base exchange data are studied.

[1] Journal Series paper of the N. J. Agricultural Experiment Station, department of soil chemistry and bacteriology.

The experiment was planned to furnish nitrogen, in equivalent amounts, from nitrate of soda, sulfate of ammonia, dried blood, and also from a mixture of the three (one-third of the nitrogen from each). The nitrogen materials were used in amounts equivalent to 320 pounds of nitrate of soda to the acre. Also, provision was made for supplying phosphorus in the form of 16 per cent superphosphate in three different amounts; namely, at the rate of 320, 640, and 960 pounds to the acre (10, 20, and 30 gm. per cylinder). Potash was used as C.P. potassium chloride to give 50 pounds of K_2O to the acre.

The arrangement of the cylinders in pairs with the fertilizer treatment is as follows:

SOURCE OF NITROGEN	SECTION	SINGLE PORTION P_2O_5	DOUBLE PORTION P_2O_5	TRIPLE PORTION P_2O_5
No nitrogen (check)	A	O O	O O	O O
Nitrate of soda	B	O O	O O	O O
Sulfate of ammonia	C	O O	O O	O O
Dried blood	D	O O	O O	O O
⅓ nitrogen from each	E	O O	O O	O O

PLAN OF RESEARCH

Soil samples were collected from the cylinders the latter part of March, 1936, and composites made of the duplicate cylinders. First, pH determinations were made on the moist samples by the electrometric method using a Leeds and Northrup glass electrode. The soils were then air-dried and put through a 2-mm. sieve. The total base exchange capacity and exchangeable hydrogen on these soils were then determined by the barium acetate extraction method. At the same time duplicate samples were extracted with normal neutral ammonium acetate solution for the determination of the individual cations.

BARIUM ACETATE METHOD FOR DETERMINATION OF EXCHANGEABLE HYDROGEN AND TOTAL CATION EXCHANGE CAPACITY

A 10-gm. sample of soil was leached on a 12.5-cm. Whatman filter paper with successive portions of normal barium acetate solution (adjusted to the neutral point) to 500 cc. A 50-cc. aliquot of the extract was then titrated electrometrically using 0.05 N ammonium hydroxide. From these data the

exchangeable hydrogen was calculated. A few cubic centimeters of barium chloride solution was then added to the extracted soil, and the excess barium washed out with water, as indicated by the absence of chlorides. The soil was next extracted with 0.05 N hydrochloric acid until free from barium (500 cc.). The barium was determined in a 100-cc. aliquot by the addition of 2 per cent sulfuric acid. From the weight of barium sulfate obtained, the total cation exchange capacity was calculated.

AMMONIUM ACETATE EXTRACTION FOR THE DETERMINATION OF CATIONS

A 25-gm. sample of soil was weighed out into a wide-mouth Erlenmeyer flask and 150 cc. of normal ammonium acetate solution (adjusted to the neutral point) added. The flasks were stoppered and shaken occasionally during the day and allowed to stand overnight. Then they were decanted on 15-cm. Whatman filter paper, and filtered into 500-cc. volumetric flasks. After being washed and decanted several times with the ammonium acetate solution, the total soil was transferred to the filter paper. The extraction was continued up to a volume of 500 cc. The extract was evaporated to dryness with 100 cc. of nitric acid, and the ammonium salts were removed by careful ignition. After the residue had been taken up with dilute hydrochloric acid, the silica was removed by dehydration in the usual manner. If iron and aluminum were present they were removed in the usual way with ammonium hydroxide. Manganese, if present, may be removed or determined by the bromine method. The solution was then made up to a definite volume, and aliquot portions were used for the determination of calcium, magnesium, potassium, and sodium.

The calcium was determined by the oxalate precipitation with subsequent titration with 0.05 N potassium permanganate.

The magnesium was determined gravimetrically as magnesium pyrophosphate.

The potassium was determined by the method of Schueler and Thomas (26). This is a volumetric method consisting of titration of the precipitated potassium cobaltinitrite with standard potassium permanganate.

The sodium was determined gravimetrically by the uranyl-zinc acetate method of Barber and Kolthoff (1).

In addition to the aforementioned determinations, organic matter was determined on these soils by the wet oxidation method of Tiurin (27).

Most of the analytical data on these soils are recorded in table 1. The averages show the effect of phosphates on the ultimate pH of a soil and on the base exchange capacity. The ultimate pH values were determined only on a composite sample of the soils for each application of phosphorus. In determining the ultimate pH, 50 gm. of the composite soil sample was electrodialyzed for about 72 hours in a Mattson cell (13). When no more bases were present in the cathodic liquid the electrodialysis was considered complete (the cathodic liquid would then be acid to phenolphthalein). The soil was removed from

the cell, dried at a temperature between 50–60°C., and then made up as a 1:2 distilled water suspension. The pH of the suspension was determined by the glass electrode method.

DISCUSSION OF RESULTS IN TABLE 1

It will be noted in table 1 that the pH values on sections A, B, and C were between 6 and 7, whereas on sections D and E, they were slightly below pH 6.0

TABLE 1

Base exchange and pH data on soils from cylinder M

(Base exchange results expressed in m.e. per 100 gm. of soil)

CYLINDER NUMBER	PO_4 APPLICATION	pH		EXCHANGE H	TOTAL CATION EXCHANGE CAPACITY		Ca	Mg	K	Na	PER CENT CARBON
		Field	Ultimate		Ba-acetate method	NH_4-acetate method					
1A, 2A	Single portion	6.25		1.54	7 24	6.14	3 90	0.45	0.13	0 12	1.55
1B, 2B	superphos-	6.48		1.32	6.81	6.11	4.26	0.30	0.09	0.14	1.63
1C, 2C	phate	6 52		1.10	7 02	5 46	3 68	0.55	0.06	0 07	1.74
1D, 2D		5 64		2.88	6 98	6 17	2 93	0.19	0 10	0 07	1.56
1E, 2E		5.70		1.98	6 68	5.17	2 73	0 29	0 10	0 07	1 53
Average		6.11	4.63	1 76	6 94	5 81	3 50	0.35	0 10	0.09	1 60
3A, 4A	Double portion	6.27		1 98	7 02	6 81	4 30	0 36	0 11	.06	1.53
3B, 4B	superphos-	6.58		1 98	6 98	5 52	3.09	0 32	...	0.13	1 56
3C, 4C	phate	6 48		2 42	7.36	7 25	4 18	0.53	0 08	0 04	1 63
3D, 4D		5.87		3.52	7.15	6.72	2 97	0 13	0 10	...	1.61
3E, 4E		5 84		3.30	7.49	6 43	2.73	0 26	0.08	0.06	1.52
Average		6.13	4.44	2 64	7 20	6 54	3 45	0 32	0.09	0 07	1 57
5A, 6A	Triple portion	6.32		3.08	8.39	6.65	2 97	0 47	0 08	0.05	1.49
5B, 6B	superphos-	6.63		3.52	8.31	7.21	3.01	0 46	0.08	0.14	1.46
5C, 6C	phate	6.48		3.52	8.01	7.31	3 38	0.26	0.10	0.05	1.65
5D, 6D		5.88		4.84	8 39	7.99	2.78	0.16	0.13	0.09	1.67
5E, 6E		6.01		4.84	7.88	8 06	2.93	0.16	0.08	0.05	1.54
Average		6.20	4.15	3.96	8.19	7.45	3.01	0.30	0.09	0.07	1.56

On section C, where ammonium sulfate was used the pH was about 6.5, due to the extra liming in 1934. Section B, or the nitrate of soda treated cylinders, showed the highest pH values, and sections D and E, the lowest. The effect of doubling and tripling the amount of phosphorus seemed to have little effect on the pH values. If anything, the effect has been to raise the pH values slightly, 0.1 or 0.2 of a pH unit. The average pH value for the single phosphate application was 6.11; for the double, 6.13; and for the triple, 6.20. These averages

were obtained by converting the individual pH values to their corresponding H-ion concentrations, and recalculating the average back to pH terms.

The exchangeable hydrogen was, however, markedly affected by the phosphate treatment. Thus, from the single portion of phosphorus added between 1 and 2 m.e. of exchangeable hydrogen was found per 100 gm. of soil; from the double portion, between 2 and 3.5 m.e., and from the triple portion, between 3 and 4.8 m.e. Each of the cylinders receiving a different form of nitrogen showed a progressive increase in exchange hydrogen as the phosphate application increased. Furthermore, the cylinders receiving the dried blood (section D) showed the greatest exchange hydrogen, probably partly because the pH values of these cylinders were the lowest.

The total cation exchange capacity by the barium acetate method showed a distinct increase when the phosphate applications were doubled or tripled. The average for the single portion was 6.94 m.e. per 100 gm. soil; for the double portion, 7.20 m.e.; and for the triple, 8.19 m.e. This fact is still more strongly emphasized in the results of the total cation exchange by the ammonium acetate method. Here the total m.e. of cations extracted were not so great as with the barium acetate method, but the differences in total exchange capacity for the three phosphate applications were more marked. The average for the single phosphate application was 5.81 m.e. per 100 gm. soil; for the double portion, 6.54; and for the triple portion, 7.45. These findings corroborate those of other investigators. Thus, for example, Merkle (17) found from base exchange studies of the Pennsylvania Jordon field plots that "the continued use of a mixture of superphosphate and muriate of potash when compared with the check plots has resulted in an increase in total exchange capacity. The fertilizer combination has decreased the replaceable calcium and increased the hydrogen content. Magnesium is unaffected but potassium is decidedly increased." Merkle states further: "Superphosphate alone caused neither increase nor decrease in calcium but did increase exchange hydrogen. This fertilizer has resulted in a diminution of magnesium and potassium which is believed to be due to larger crop removals than the check plots. Superphosphate increases soil acidity."

The ultimate pH value of the composite soil samples from cylinders receiving single, double, and triple amounts of phosphorus, may be compared to the field pH, total cation exchange, and exchange hydrogen. The drop in ultimate pH as the amount of added phosphate increases is well correlated with the corresponding increase in total cation exchange and exchange hydrogen.

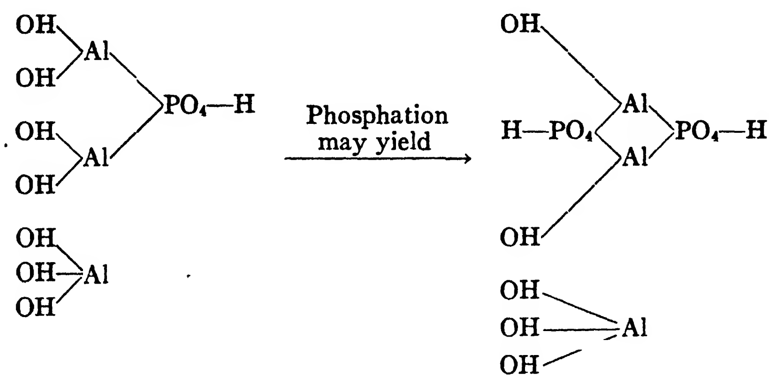
These facts, especially the drop in ultimate pH value, further support Mattson's (16) contention that phosphates alter the acidoid-basoid ratio by increasing it, and as a result increase the total cation exchange capacity. Mattson has further shown that silicates and humates as well as phosphates act as acidoids (14), and any change in the amount of such substances effect a change in the acidoid-basoid ratio.

$$\frac{SiO_2,\ \text{humus},\ P_2O_5}{R_2O_3} = \frac{\text{acidoid}}{\text{basoid}}$$

It must be borne in mind, however, that the relation between the introduction of new acidoid constituents into the soil colloidal complex, and the increase in cation exchange capacity, is not a simple linear function. According to Mattson, the variations in the acidoid-basoid ratio express only one of several factors which greatly influence the behavior of the soil colloid.

The following structural formulas, taken from Mattson's studies, illustrate the alteration in the cation exchange capacity which may occur upon phosphation of a synthetic aluminum phosphate complex. Silicates or humates might enter the complexes in a similar way. No doubt soil colloids behave in a like manner.

I II



$$\frac{P_2O_5}{Al_2O_3} = 1/3 \longrightarrow \frac{P_2O_5}{Al_2O_3} = 2/3$$

In the case of complex number II the quantity of displaceable hydrogen is increased and displaceable hydroxyl ions decreased, indicating an increase in cation exchange capacity and a marked reduction in anion exchange capacity.

From these considerations the question may arise as to the influence of the organic matter content of these soils on the cation exchange capacity. It will be noted in the last column of table 1 that the organic matter content of the individual cylinders is rather uniform and shows no marked variation. Hence, in the case of these soils, the increase in total cation exchange capacity cannot be attributed to an increase in organic matter.

The individual cations, calcium, magnesium, potassium, and sodium, as determined by the ammonium acetate method, showed no difference in the amounts exchangeable when single, double, and triple amounts of phosphorus were added. Apparently it is only the amount of exchangeable hydrogen which is affected by increased phosphation on these soils. Further addition of alkali and alkaline earth cations to these soils would probably mean a greater adsorption by those soils receiving the most phosphates. With those soils where the pH was above 6.0, the exchangeable calcium and magnesium were greatest. The exchangeable potassium was very constant for all of the cyl-

inders, about 0.1 m.e. per 100 gm. of soil. Also the exchangeable sodium was very low, but it is interesting to note that the amount on the nitrate of soda treated cylinders was about twice as much as on the other cylinders. No exchangeable manganese was found on these soils.

INFLUENCE OF PHOSPHATION UPON THE CATION EXCHANGE CAPACITY OF A CALCIUM-SATURATED COLTS NECK LOAM

Evidence that phosphation of soil material increases the cation exchange capacity is further shown by the results of the laboratory experiment summarized in table 2. This work was carried out as follows: One hundred gram portions of a calcium-saturated Colts Neck loam soil were suspended in 500 cc. quantities of distilled water each containing increasing increments of P_2O_5 as di-sodium phosphate and allowed to stand overnight. The following

TABLE 2

Influence of phosphation upon the cation exchange capacity of a calcium-saturated Colts Neck loam

TREATMENT—P_2O_5 AS Na_2HPO_4	ANALYSIS OF SUPERNATANT LIQUID				ANALYSIS OF NH_4Cl EXTRACT		CATION EXCHANGE CAPACITY BY N NEUTRAL NH_4Cl	INCREASE IN CATION EXCHANGE CAPACITY	P_2O_5 HELD BY 100 GM. SOIL	IRON IN THE SOIL AS FREE Fe_2O_3
	pH	Humus	SiO_2	CaO	CaO	P_2O_5				
		mgm	*mgm.*	*m.e.*	*m.e.*	*milli-moles*	*m.e. per 100 gm*	*m.e. per 100 gm.*	*milli-moles*	*per cent*
Ca-soil*...	6 95				15.38	trace	15 08			11.92
5 millimoles P_2O_5	8 05	150	2 5	0 61	15.08	0 5	18 12	2 95	2 9	11 56
15 millimoles P_2O_5 ...	8 10	493	0 5	0.51	12 71	1.7	18 54	3 37	5 5	10 92
25 millimoles P_2O_5	8 15	583	3 0	0.48	10 80	2 3	19.39	4 22	7 3	9 97

* 1:2 water suspension.

day the supernatant liquid was filtered off and the soils were dried at a temperature between 50 and 60°C. The soils were then allowed to cool at room temperature. The cation exchange capacities of the soils were determined in triplicate on 10-gm. samples by the normal neutral ammonium chloride method. Total P_2O_5 determinations on the original calcium-saturated soil and on the phosphated calcium soils yielded the values for the quantity of P_2O_5 held by the soil. Free Fe_2O_3 was determined by the method of Drosdoff and Truog (7). Displaced organic matter in the supernatant liquid is expressed in terms of humus following the method of Tiurin (27).

An alteration in the nature of the exchange complex results upon phosphation and is evident from the data on the analysis of the supernatant liquid and the values for the cation exchange capacities as seen in table 2. Appreciable quantities of humus appeared in the supernatant liquid during phosphation, a fraction of which undoubtedly is due to increased solubility of the organic matter with increasing pH. The increase in humus cannot be entirely ascribed, however, to the forenamed effect, especially among the three phosphate

treatments, since in these cases a difference of only 0.1 pH unit exists in the three treatments and yet the data indicate a rise from 150 to 583 mgm. of humus in the supernatant liquid. The release of humus with increasing phosphation is in part due to a displacement of the humate fraction of the soil colloidal complex by phosphates (14). The low and irregular values for silica in the superantant liquid probably have no significance.

The quantities of phosphorus held by the soil include both a fraction loosely held by the soil and a fraction fixed by the free ferric oxides and possibly by exchanged calcium. It was to be expected, therefore, that the ammonium chloride treatment would extract some of the retained phosphorus. The data on the analysis of the ammonium chloride extract bears out this point; the extracted phosphorus values ranged from 0.5 to 2.3 millimoles of P_2O_5 from the lowest to the highest treatments of P_2O_5.

The exact behavior of the exchangeable calcium during phosphation cannot be followed with any degree of certainty from the data recorded in table 2. One point, however, is quite apparent, namely, that calcium is fixed during phosphation in the two higher phosphate treatments. This is illustrated by the displacement of only 12.71 and 10.80 m.e. of calcium oxide out of a total of 15.38 m.e. by the ammonium ion. The fixation of calcium may be due to the formation of calcium phosphate complexes (19). The behavior of the exchangeable calcium during the phosphation process is being further studied.

The retention of approximately 2.4 millimoles of P_2O_5 (millimoles P_2O_5 held minus millimoles P_2O_5 extracted by ammonium chloride) has increased the cation exchange capacity (at pH 7.0) by 2.95 m.e. Increasing retention of P_2O_5 has raised the cation exchange capacities by 3.37 and 4.21 m.e. with the two higher phosphate treatments. Undoubtedly, these recorded increases in the cation exchange capacity are not due entirely to phosphation, since an increase in pH generally increases this property (5). Nevertheless, the increase due to higher pH values must be compensated in part by the loss of humus, which has a high cation exchange capacity (a sample of humic acid prepared by one of the authors had a cation exchange capacity of 274 m.e. per 100 gm.). Whether or not the loss in exchange capacity due to the mobilization of organic matter exceeds the increase due to the rise in pH cannot be stated with certainty. The fact remains, however, that phosphation has increased the cation exchange capacity.

A parallelism between the increase in cation exchange capacity and a reduction in free ferric oxides is to be noted in the table. It is possible that new complexes of iron and phosphorus have been formed, and the increase in cation exchange capacity may be due in part to the exchange properties exhibited by these combinations.

SUMMARY

Base exchange studies were conducted on Sassafras silt loam soil which had received three different amounts of superphosphate yearly since 1922 and had received equivalent amounts of nitrogen from different sources.

Two extraction methods were used in determining the exchange hydrogen, the total cation exchange capacity, and the individual cations. Also several composite soil samples were electrodialyzed to determine the ultimate pH values.

The following important points were brought out by the analysis of these soils:

The field pH values were very little affected by increasing the amount of phosphates.

The exchangeable hydrogen was greatly increased by phosphation, and the ultimate pH values were lowered.

The total cation exchange capacity was distinctly increased when the phosphorus applications were doubled or tripled.

The above findings were very similar to those obtained by Merkle on the Pennsylvania Jordan field plots. They may be explained on the basis that phosphates increase the acidoid-basoid ratio. As a result, the total cation exchange capacity increases.

The exchangeable calcium, magnesium, potassium, and sodium showed no significant differences in relation to the amount of added phosphates.

Further evidence that phosphation of a soil material increases the cation exchange capacity was shown by a laboratory experiment using a calcium-saturated Colts Neck loam. With this soil, phosphation increased the cation exchange capacity depending upon the amount of phosphorus adsorbed. Also a correlation between the increase in cation exchange and the reduction in free ferric oxide was noted in this study.

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AVAILABILITY OF SOIL PHOSPHATES FOR THE PLANT FROM THE VIEWPOINT OF COLLOID CHEMISTRY

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In this paper the question of the availability of soil phosphates is considered from the standpoint of the chemistry of colloids, only the peculiar character of the fixation of phosphates in the soil, as a phenomenon of adsorption, and that of the "mobility" of the fixed phosphates, as the opposite phenomenon of "desorption,"[1] being taken into account. We know, of course, that the fixation of phosphates by soil and the opposite process, the so-called "mobility" of phosphates, may be considered from a purely chemical point of view, and so it is actually often dealt with in the literature.

If we consider the fixation of soluble phosphates, such as superphosphate and ammonium phosphate, from the colloid chemical viewpoint, the whole process should be explained in terms of adsorption of phosphate anions by soil gels. The task would be easier if the gels were uniform, for instance in their electrokinetic properties, within the limits of the same soil at least. The electrokinetic properties are mentioned because they are, as Mattson (15) and other authors, e.g., Gile (7), have demonstrated, very important for the adsorption of oppositely charged ions, but in reality there is no such uniformity in the the same soil or even in the same horizon. To the contrary, gels in one soil can be negative in one part and isoelectric in another (19, 21). The possibility is not to be excluded of the existence in soil of not only the two first groups of gels, but also of a third one, charged positively, as the existence of free sesquioxides in soil has been proved by a number of authors, e.g., Kerr (11), Ross (17), Denison (4). It is interesting to note, that Kubiena (12) was able to point out directly under the microscope both the motley character of soil colloids and the presence of areas of sesquioxides in this motley picture. Under the conditions of reaction commonly prevailing in soil (pH 7.0) the free sesquioxides will be charged positively.

Since the chief rôle in the fixation of soluble phosphates by soil is ascribed

[1] "Desorption" is the same thing as adsorption, only in "desorption" our attention is fixed on only those ions which, because of one or another adsorption process, pass into solution from the double layer. In other words, in some kinds of adsorption, while one ion enters the double layer and is fixed in it, another one is liberated from it. Such an ion, liberated from the double layer, we call a "desorbed ion," and the process itself, of the passage of the ion into solution, we name "desorption."

to the sesquioxides, our investigation began with a study of these. This was done in the work of Shtatnov and Odintzova in 1934 (18) under the guidance of the author.

The process of fixation of soluble phosphates in soil by means of adsorption—had been already demonstrated. We were interested in the opposite process, that of "desorption" under conditions of varying degrees of saturation of the iron gel by the phosphate anion. This phenomenon might have been studied either by displacing the phosphate anion by other anions under varying conditions, as was done by Demolon and Bastisse (3), or directly in the plant under given conditions. We chose this second method.

A freshly prepared iron gel was saturated to different degrees with phosphate anions from solutions of varying concentrations of monosodium phosphate (NaH_2PO_4). The availability of these adsorbed phosphate anions to the plant (oats) was then tested directly, all other conditions being equal. The technique used for saturation is very important in this case. The aim is to get uniform distribution, and the following procedure was adopted: Large volumes of solutions of varying concentration of NaH_2PO_4 were prepared in large glass cylinders. Iron gel (or soil) was then poured into the solution and carefully mixed. When the entire gel had settled and the solution over the precipitate had become clear, the solution was pound off and the gel transferred into a parchment sack for dialysis. After dialyzation, the gel was dried at 30°C. and tested for P_2O_5.

The results of the analyses are summarized in table 1. As may be seen from this table, three degrees of saturation were obtained. The strongest solution produced a lower degree of saturation than the medium one. We are unable to give a full explanation of this phenomenon; we can offer only a theory. The electrokinetic properties, determined by a rough qualitative test (by means of electrophoresis), proved different in all three cases. The iron gel in the lower degree of saturation remained positively charged; after saturation from the 0.1 *M* phosphate solution it became isoelectric, and after saturation from the strongest solution —0.25 *M*—it became negatively charged. It is possible that the high content of adsorbed phosphate anions after saturation from the 0.1 *M* solution is somehow related to the isoelectric condition of the gel.

The material which was to serve as the source of phosphate nutrition for the plants was supplied in an isolated state, not as a part of some general nutrient mixture. The inner container into which one part of the roots was placed was filled with Helriegel's nutrient mixture but without phosphates, whereas the outer container, into which the remaining roots were put, contained only phosphates mixed with pure quartz sand. With this arrangement, only the organic anions liberated by the plant itself would displace the phosphate anion from the double layer. In other words, we feared that other anions of the nutrient mixture might increase the availability of the adsorbed phosphate anions to the plant. It appeared in the course of this investigation, however, that these fears were groundless. In those cases where the solution from the inner con-

tainer passed into the external one, the phosphate anions at a low degree of saturation were also unavailable to the plant, in whatever amount they were supplied.

Table 2 shows the results of the experiments with the iron gel. The availability of the adsorbed phosphate anions, under the conditions of our experiment, increases with the degree of saturation of the gel. Moreover, below a certain degree of saturation, the phosphate anion is totally unavailable to the plant, whatever the amount applied. We have called the degree of saturation below which the adsorbed anions remain unavailable to the plant the "critical zone of saturation." Its great practical significance was pointed out. A

TABLE 1

Analyses of dialyzed phosphate gels

GEL NUMBER	CONCENTRATION OF NaH_2PO_4 SOLUTION	RATIO OF SOLUTION TO GEL	P_2O_5 ABSORBED PER GM. OF GEL	pH OF SUPERNATANT LIQUID (MICHAELIS METHOD)	pH OF RESPECTIVE NaH_2PO_4 SOLUTION
	M		*mgm.*		
1	0 25	300:1	16 72	5 1	4.5
2	0.1	300:1	21 12	5 9	4.8
3	0.001	500:1	3.23	7.0	5.5

TABLE 2

Yield of dry matter with variously saturated gels

(gm per container)

P_2O_5 PER CONTAINER	FREE NaH_2PO_4	P_2O_5 FIXED BY IRON GEL (MGM. P_2O_5 PER GM. OF GEL)		
		3 238	16.726	21.12
mgm.	*mgm.*			
10	5 34	3 51	3 75	3 68
30	9.51	4 06	5 67	6 28
80	18 51	3.22	10 38	15.80
160	20.74	5 89	17.24	....

knowledge of the phenomenon of the "critical zone of saturation" may permit the computation of the quantity of fertilizer to be added to the soil in question. The particular significance of this was pointed out in an earlier paper (20).

In 1935 the Soil Colloid Laboratory of the All-Union Institute of Fertilizers inaugurated experiments directly on soil, instead of, as first planned, on the availability of adsorbed phosphate anions from soil gels with varying electrokinetic properties. S. V. Odintzova and A. N. Lawrienko worked on pot-culture experiments on krasnozem (red soil), and E. M. Rosenfeld, on chernozem from which Ca had been removed. The soils were, however, so selected as to reflect to a certain degree the original plan: krasnozem was to imitate gels of the sesquioxide type which we already had studied in pure condition, and

chernozem was to imitate negatively charged gels. To avoid complications which might be caused by the calcium cation, we eliminated it from chernozem by the method proposed by Gedroitz, i.e., by treatment with 0.05 N HCl and subsequent neutralization with weak solutions of sodium hydroxide.

TABLE 3

Analytical results on soil experiments

SOILS	RATIO OF SOLUTION TO SOIL	CONCENTRATION OF NaH_2PO_4 SOLUTION	P_2O_5 CONTENT PER GM. OF SOIL
		M	*mgm.*
Original soil	...	...	1.18
Saturated	200:1	0.01	16 0
Saturated	200:1	0 25	25 93

TABLE 4

Crop yields on krasnozem

(gm. per container)

P_2O_5 PER CONTAINER	DRY MATTER ON ORIGINAL SOIL: 1.18 MGM. P_2O_5 PER GM. OF SOIL	P_2O_5 PER CONTAINER	DRY MATTER ON	
			First degree of saturation: 16.0 mgm. P_2O_5 per gm of soil	Second degree of saturation: 25.93 mgm. P_2O_5 per gm. of soil
mgm.	*gm.*	*mgm*	*gm.*	*gm.*
5	3.22	10	3 59	7 0
15	2.98	30	5 05	7.37
25	3.15	50	9 36	9 77
40	2.86	80	12 72	12 80
75	2.95	150	12.70	16 22

TABLE 5

Crop yields on chernozem

(75 mgm. P_2O_5 per container)

DEGREE OF SATURATION OF CHERNOZEM	AVERAGE YIELD PER CONTAINER
	gm.
Original soil, 1.6 mgm. P_2O_5 per gm. of soil	3 06
Ca-free soil, 0.49 mgm. P_2O_5 per gm. of soil	3.15
Ca-free soil, saturated 2.4 mgm P_2O_5 per gm. of soil	6.33
Ca-free soil, saturated 3.54 mgm. P_2O_5 per gm. of soil	9.33

The krasnozem was brought from a tea plantation in one of the humid subtropical districts of U.S.S.R. (Chakva, sample taken from fallow land overgrown by ferns). The soil was screened through a 0.25-mm. sieve and then placed into large cylindric containers (40 l.) filled with solutions of the same phosphate (NaH_2PO_4) in varying concentrations. After this it was continu-

ously and carefully mixed for 2 days to issue uniform saturation. The rest of the procedure was the same as in the experiments with the iron gels: pouring off of the supernatant liquid after sedimentation, dialization of sediment, and testing for P_2O_5.

The results are summarized in table 3. As this table shows, two different degrees of saturation were obtained.

From 5 to 150 mgm. of P_2O_5 were then introduced into the external container, with the results shown in table 4. As was observed in experiments with the iron gel, here also the higher is the degree of saturation of krasnozem, the higher will be the availability of the adsorbed phosphate ions. There is also a critical zone, which, however, we did not try to determine more precisely in this case. It is clear only that the degree of saturation of the original soil with phosphate anions lies below this critical zone.

In Rosenfeld's experiment with the Ca-free chernozem, the original chernozem contained 1.6 mgm. P_2O_5 per gram of soil; after removal of Ca, the chernozem contained only 0.49 mgm. P_2O_5. Saturation from a 0.005 *M* solution, using 50 volumes of the solution to 1 volume of soil, raised the content of P_2O_5 to 2.4 mgm. per gram of soil, and from the 0.01 *M* solution, to 3.54 mgm. It must be noted that saturation of chernozem from higher concentrations than 0.01 *M* did not increase the degree of saturation; on the contrary, the curve of saturation turned downward. The causes of this phenomenon were not investigated.

The results of the experiment with chernozem are summarized in table 5. As can be seen, here also there is a critical zone of saturation, below which the phosphate anions are unavailable and above which they are available, the degree of availability rising with the degree of saturation.

Parallel with the application of 75 mgm. P_2O_5 per container, an application of 125 mgm. P_2O_5 per container was tested. The results obtained were the same: at the low degree of saturation, the larger application of P_2O_5 remained as unavailable to the plant as the smaller one, and above the critical zone, the yield increase was proportional to the degree of saturation.

DISCUSSION

In the experiment for the determination of the availability of adsorbed phosphate anions to the plant, if careful precautions are taken to obtain uniform saturation of the iron gel or soil, an evident dependence of this availability on the degree of saturation of the gel or soil with the phosphate anions is observed. This has been demonstrated by our experiments; was predicted earlier on theoretical grounds by Gaarder (6), Demolon and Barbier (2) et al.[2]; and later was indicated also by Hibbard (9). It is still not clear, however, with what type of adsorption we are dealing. On the basis of recent work, three

[2] Gordon seems to be the first to have done work in this direction on plants. We did not have access, however, to his work in the original (WILEY, R. C., AND GORDON, N. E. 1923 Availability of adsorbed phosphates. *Soil Sci.* 15: 371–373).

types of absorption may be distinguished, as indicated by Verwey (22): (*a*) adsorption according to Gibbs; (*b*) exchange of counter-ions (the ionic spheres of opposite charge around the colloid-ions—charged particles); (*c*) assimilation of potential-determining electrolytes.

The first type of adsorption has but limited importance for electrolytes in soil.

The second type—exchange of counter-ions—is very important in soil for the cation exchange in negatively charged gels. As for anions, this kind of adsorption is applicable only to positively charged gels. Our experiments show, however, that phosphate anions are least available for the plant when the iron gel retains its positive charge. Only after the iron gel has passed into the isoelectric condition, particularly on the negative side, do the adsorbed phosphate anions become available to the plant. Hence the necessity follows of grounding theoretically the desorption of phosphate anions from the isoelectric or electronegative soil gels.

The adsorption of phosphate anions might be explained with the aid of the assimilation of potential-determining electrolytes. In this case one of the ions is included in the crystal lattice and it determines the sign of the colloid charge and the potential. The phosphate anion would charge the isoelectric colloid negatively. The other ion, in our case the cation, would occupy its place in the external layer. But such an assumption makes it difficult to understand the opposite process, that of the desorption of phosphate anions. Indeed, to make it possible for the negative ion to break through the surface of the crystal lattice, which is also negatively charged, it must possess not only corresponding dimensions, but also a very high energy of activation. Even some of the phosphate anions satisfy these conditions, they do not suffice for the desorption of the said ions. For the desorption of phosphate anions it is necessary that other anions, e.g., the anions of organic acids, satisfy these conditions. Only under these circumstances would the phosphate anions be able to pass from the crystal lattice into the surrounding solution and become available to the plant. But such an assumption is improbable. It seems more expedient, therefore, to accept for the explanation of the desorption of phosphate anions from negatively charged gels the theoretical considerations of Kargin concerning molecular adsorption. Kargin in his reports[3] on molecular adsorption has demonstrated that purified ammosilicates adsorbed both ions of the electrolyte from the solution in equivalent quantities. If, after this, electrolytes are introduced into the solution, a new equilibrium is established, the result of which will be adsorptional distribution. This means that the ions of the new electrolyte will enter the double layer in pairs, and from it a part of the adsorbed phosphate anions will pass into the solution. Kargin has shown, besides, that the lower the solubility of the respective salt, the higher the molecular adsorption (in the case of silver salts). It is probable that this rule will hold true also in respect of phosphates of various solubilities.

[3] Unpublished.

From the work of Kargin, it is possible to explain both adsorption and desorption of phosphate anions without resorting to the phenomenon of penetration of these ions into the crystal lattice of negatively charged soil colloids. Both adsorption and desorption of phosphate anions will take place in the double electric layer of the negatively charged gels at the surface of the colloid particle. This does not mean that molecules, as such, are adsorbed in this process. Adsorption in the ion form is more probable. At first, as a result of affinity and high energy of activation, a certain quantity of phosphate anions, in spite of the force of repulsion exerted by the negative colloid on the anion, will enter the double layer and approach definite areas of the crystal lattice. But then the electric properties of the double layer will suffer an alteration: the electrokinetic potential will be raised. This will lead to the entering of equivalent amounts of cations from the solution, and adsorption will become molecular. This is the manner in which adsorption of phosphate anions would proceed, according to Kargin. When new electrolytes, such as organic acids, are introduced into the solution, the anions of these acids and their respective cations will enter the double layer in the manner already described. But since this double layer is already saturated, the phosphate anions, together with a part of the cations, will pass into solution. This will be desorption, as the result of adsorptional distribution of ions.

The results of Mattson (16) on the displacement of silica anions from negatively charged colloids with the help of, or by means of, phosphate anions, as well as the results of the experiments of Demolon and Bastisse (3) on the displacement of phosphate anions from soils by the anions of various acids, can also be explained from this standpoint.

Future work on this problem may well be developed along one of the following lines.

Our idea that each soil possesses its own critical zone of saturation might be used for determining quantitatively, at least approximately, the fertilizer requirement of the soil, particularly in regard to phosphates. This method is preferable to the cumbersome pot-culture method. A method must be elaborated for the determination of the limit of the degree of saturation, which may be called provisionally "the soil absorption capacity," with respect to phosphate anions. Of course, this value should be determined only under definite conditions of pH, of concentration, of ratio of soil to the amount of the salt solution, and of time of contact. But this will not impair its practical value.

Many attempts have been made to determine the reserves in the soil of phosphoric acid available to the plant (2, 5, 8, 9, 13, 14, 22) but they were concerned mostly with solubility. Of these, the most pertinent to our work is that of Demolon et Barbier (2). Particularly valuable are the results they obtained with citrates, though the authors themselves are not certain about their method. They say that more phosphoric acid passes into the citrate solution than into that of acetic and nitric acid, because the citrate destroys

the adsorbents in the soil. Lohse and Ruhnke seem to us to be nearer the truth when they indicate (13, p. 450) that such acids as acetic and nitric liberate not only the phosphoric acid but also other ions, such as that of iron, and the latter precipitates the phosphate anion. This is eliminated in citrate treatment, as here a complex compound is formed. In short, we think that the citrate method of Demolon et Barbier might supply interesting results if it were modified and adapted to the aims we are pursuing.

It is possible that microbiologic methods might prove both easier and more suitable for determining the availability of phosphoric acid to the plant, but this is outside the realm of this treatise.

It was not our intention to deal here with the problem of the means of lowering the level of the critical zone of saturation, which is of great practical importance. Such a lowering may be achieved, however, by means of liming or introduction of silica, as has been indicated by Gaarder (6, p. 41), or by the introduction of organic fertilizers. This subject will be dealt with in a special article.

SUMMARY

An attempt has been made to study the availability of adsorbed phosphate anions to the plant from the standpoint of colloidal chemistry.

The gel of iron and two soils—krasnozem and chernozem—were used as absorbents.

Both the iron gel and the soils were evenly saturated with phosphate anions to varying degrees of saturation by means of a special method.

The direct availability of the adsorbed phosphate anions to the plant (oats) was tested in pot-culture experiments (with isolated nutrition) in relation to the degree of saturation.

The phosphate anions became available to the plant at the critical zone of saturation. Below this zone the phosphate anions are unavailable to the plant, even when supplied in quantities for exceeding the needs of the plant.

In explaining the availability or desorption of phosphate anions we have mainly utilized the theoretical considerations of Kargin, which follow from his recent, unpublished work on molecular adsorption and adsorptional distribution.

The practical significance of the critical zone of saturation for the quantitative determination of the fertilizer requirement of the soil is indicated.

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PHYSIOLOGICAL STUDIES ON RHIZOBIUM: VII. SOME PHYSIOLOGICAL EFFECTS OF ACCESSORY GROWTH FACTORS[1]

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In a previous paper of this series (7) it was shown that the rhizobia are able to grow continuously with frequent transfers in properly balanced media made up of c.p. materials. No evidence was found that these organisms require any complex unidentified substances for either growth or respiration. Allison and Hoover (1, 2, 5), however, have shown that certain extracts greatly facilitate the growth of the rhizobia in a synthetic (mineral-nitrate-sucrose) medium. The accessory substances in these extracts were found also to stimulate the growth and oxygen utilization of these organisms in similar media containing nitrogen sources other than nitrates.

Allyn and Baldwin (3, 4) showed that some reducing agents such as cysteine and thioglycollic acid in certain concentrations greatly increased the growth of the rhizobia in a mineral-nitrate-mannitol medium. Thorne and Walker (7) found that cysteine induced increases in the growth and respiration of the rhizobia comparable to those brought about by brown cane sugar which contains appreciable quantities of accessory factors. Such results seemed to suggest that the explanation of the ability of the accessory growth substances studied to stimulate these organisms might be associated with a reducing effect, tending to bring about a more favorable potential in the medium for growth. The present investigation was planned to study some of the physiological effects of these stimulative materials upon the rhizobia.

PROCEDURE

It has been found very convenient to study the influence of the various stimulative substances upon the rhizobia by the determination of the gaseous exchange of the organisms in the presence of these materials. The net oxidative and reductive changes of organisms can be conveniently expressed by their respiratory quotients. The respiratory quotient is the value obtained from the ratio,

$$\frac{\text{Volume } CO_2 \text{ produced}}{\text{Volume } O_2 \text{ consumed}}$$

[1] Journal paper J-360, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 226.

[2] Research fellow and formerly research associate professor of soils, Iowa State College, respectively. The authors are indebted to Dr. P. E. Brown for the suggestions and criticisms offered in the course of this work and in the preparation of the manuscript.

In general the respiratory quotient of bacteria seems to be influenced by two major factors: first, the chemical composition and physical characteristics of the medium in which the organisms are grown, and, second, the natural or innate oxidation-reduction characteristics of the organisms.

Oxygen consumption and CO_2 production of the rhizobia were determined with Warburg manometers according to the direct method of Warburg (8). A special absorption coefficient for CO_2 was determined for the medium used under the pressure range encountered in the various studies. This coefficient was substituted in Warburg's equation and used in calculating the CO_2 vessel constant for each of the respirometer vessels used without alkali in the inner well. Although this method is not applicable to all microörganisms, a number of experiments have shown it to be very accurate for the rhizobia. It has the advantage that continuous readings of both oxygen and CO_2 can be made, whereas, with other methods CO_2 production is determined only at the end of the experiment. The method is adaptable to these organisms because of their aerobic nature and also because they bring about no appreciable change in the pH of the medium during such short periods as those in which the determinations were made.

The following basal medium was employed throughout the experiments reported: K_2HPO_4, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.1 gm.; $CaCO_3$, 3.0 gm.; $FeCl_3$, 0.01 gm.; distilled water, 1,000 cc. The sugars employed were added to give a concentration of 1 per cent; the fixed nitrogen content of each medium was made up to 100 p.p.m. The preparation of inocula and the procedure with the Warburg respirometers were the same as in the previous work (6, 7).

Strain 206 of *Rh. trifolii* and 132 of *Rh. meliloti* of the laboratory stock cultures were used for the various studies. Most of the data reported are for *Rh. trifolii*, similar results were also obtained with cultures of the alfalfa organisms.

EXPERIMENTAL

In the first experiments of this series the organisms used for inoculum were prepared by culturing in yeast extract medium. The media employed in the tests and the oxygen consumption and respiratory quotients of the cultures of *Rh. trifolii* during 16 consecutive hourly intervals are given in table 1.

The data of table 1 show that cysteine, soil extract, brown cane sugar, alfalfa extract, and yeast extract each led to a distinct reduction in the value of the respiratory quotient of the organisms when compared to the quotient of the control culture growing in the KNO_3-sucrose c.p. medium. The respiratory quotients of the cultures in the media to which cysteine, soil extract, or brown cane sugar had been added increased rapidly after growth of the organisms had begun. It seems probable from these results that the stimulative substances studied contain materials acting as reducing agents or initial hydrogen donators for the organisms. The reducing substances contained in

these materials were apparently rapidly utilized. This was indicated by the comparatively short time in which the values of the respiratory quotients of the cultures in the presence of these substances rose to about the same value as that of the respiratory quotient of the control culture. Yeast extract and alfalfa extract were present in greater concentrations than the other accessory factors and also exerted a reducing effect upon the quotient over a longer period

TABLE 1

Oxygen consumption and respiratory quotients of Rh. trifolii during consecutive hourly intervals in media containing different substances

Organisms for inoculum cultured in yeast extract medium

KNO_3* + SUCROSE C.P.						KNO_3* + BROWN CANE SUGAR		ALFALFA EXTRACT* NO. 4 + SUCROSE C.P.		YEAST EXTRACT* + SUCROSE C.P.	
Control		Cysteine†		Soil extract‡							
O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.
cu. mm.		*cu. mm.*		*cu. mm.*		*cu. mm.*		*cu. mm.*		*cu. mm.*	
20.9	1.04	18.1	0.95	37.2	0.77	33.3	0.84	39.6	0.78	37.7	0.64
33.5	1.09	45.5	0.87	66.2	0.83	52.5	0.85	90.9	0.77	85.8	0.85
37.4	1.08	62.7	1.01	62.8	0.92	54.4	0.94	105.5	0.83	103.8	0.90
40.2	1.13	74.3	1.04	55.8	0.96	58.9	1.01	116.1	0.86	102.4	1.03
43.6	1.13	83.7	1.07	58.3	0.93	73.1	1.08	110.6	0.92	117.2	0.97
48.2	1.12	88.5	1.03	59.4	0.98	87.2	1.15	130.6	0.91	134.7	0.95
42.8	1.16	89.4	1.05	58.6	1.02	90.2	1.15	112.6	0.92	110.0	0.99
43.8	1.13	85.5	1.02	57.1	1.04	85.5	1.16	109.6	0.93	94.1	1.02
45.9	1.08	97.1	1.05	61.1	1.00	97.7	1.15	104.9	0.93	95.4	0.97
50.3	1.10	108.5	1.08	64.5	1.04	112.7	1.15	80.7	0.97	93.8	0.99
47.7	1.11	116.4	1.08	61.4	1.08	121.5	1.15	72.6	0.94	85.9	1.01
50.1	1.13	120.8	1.13	63.0	1.12	132.0	1.17	71.5	0.92	80.5	1.04
52.4	1.10	133.4	1.10	68.2	1.07	148.7	1.16	67.7	0.98	85.7	1.00
53.0	1.10	119.7	1.16	63.3	1.11	155.2	1.23	67.5	0.94	77.3	1.02
51.0	1.14	101.3	1.18	72.1	1.12	151.8	1.21	63.5	0.94	76.9	1.02
55.4	1.10	84.4	1.22	74.1	1.11	132.5	1.20	59.1	1.01	71.2	1.02
716.2	1.11	1,429.3	1.08	983.1	1.02	1,587.2	1.14	1,403.0	0.92	*1,452.4*	*0.97*

* KNO_3, alfalfa extract, and yeast extract in concentration of 100 p.p.m. of N.

† Cysteine 0.002 per cent.

‡ Soil extract 1 gm./liter.

of time than was observed for the other materials. Yeast and alfalfa extracts are composed largely of protein materials having theoretical respiratory quotients near 0.80, and are also readily available nutrient sources for the rhizobia. The low respiratory quotients of *Rh. trifolii* in media containing these proteinaceous extracts might, therefore, be explained as due to the organisms oxidizing considerable of these materials, which are more reduced than sugars, for their source of nutrition and energy.

An investigation similar to that reported in table 1 was conducted using inocula freed from growth substances by culturing the organisms for several transfers in KNO_3-cane sugar medium. An alcoholic extract of *Az. vinelandii* medium, prepared according to the method of Hoover and Allison (5) was investigated in addition to the materials studied in the previous experiment.

TABLE 2

Oxygen consumption and respiratory quotients of Rh. trifolii during consecutive hourly intervals in media containing different substances

Organisms for inoculum cultured in KNO_3 medium

KNO_3* + SUCROSE C.P.								KNO_3* + BROWN CANE SUGAR		ALFALFA EXTRACT* NO. 4 + SUCROSE C.P.		YEAST EXTRACT* + SUCROSE C.P.	
Control		Cysteine†		Soil extract†		Az. vin. extract‡							
O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q	O_2	R.Q.
cu. mm.		*cu. mm.*		*cu. mm.*		*cu. mm.*		*cu. mm.*		*cu. mm.*		*cu. mm.*	
8 0	1 24	8.5	0.89	13.7	0.83	5.2	0 95	11.6	1.01	13.1	1.00	15.6	0.90
8.4	1.13	8.5	0.75	17.4	0.83	9.3	0 90	12.7	1.04	17.3	0 92	13 8	0.88
9.2	1 12	8.8	0.78	23 6	0 84	13 7	0 84	15.9	1.01	22.1	0.95	23.4	0.86
7.3	1.10	9.0	0.85	24.2	0 87	14 6	0 90	18.7	1 04	28.3	0 95	26.9	0.91
6.9	1.11	9.6	0 86	24 5	0.93	13 1	0 82	18.7	1 08	29 2	0 94	32 9	0.91
7.1	1.05	10.0	0 92	26 7	0 94	11.7	0.95	20 1	1.10	35 6	0 93	43 5	0.98
6 3	1.10	10 9	0 96	27.9	0 93	11.1	0 93	21 8	1 10	32 2	0 94	45 9	0.98
7.1	1.10	10.6	0.98	28.2	0 96	11.6	0 93	24 9	1.07	45.1	0.92	54 5	0.93
7.1	1.06	10 5	0 95	31 3	0 96	11.4	0 96	29 7	1 10	50.7	0.91	61.0	0.91
6 4	1.08	10.5	0 94	31.3	0.96	11.9	0 95	34 6	1 15	55 7	0.90	65.0	0.89
5.1	1.07	10 3	0 95	31 3	0.96	10 6	0 94	36 8	1.17	57.0	0.87	65.6	0.88
4 5	1.10	10 0	1 03	33 6	0.96	10 1	0 98	40 5	1 18	58.6	0 88	66 2	0.87
4.3	1.09	10.1	1.04	34.1	0 97	10.1	1.00	46.4	1 21	69 2	0.87	70 3	0.87
5.1	1.08	10 3	0.98	35.6	0.98	9 3	0 96	51.3	1.20	68.4	0 87	74.6	0.87
5.3	1.10	9.9	0.96	36.9	0.92	9.6	1.01	51.5	1.20	70 6	0.89	71.0	0.89
4.3	1.09	9.3	0.95	33.2	0 91	7.5	1.07	57.8	1 15	77.9	0.91	70.8	0.88
102 4	1.12	156.8	0.95	453.5	0.93	170 8	0.93	493 0	1.15	731.0	0.90	801.0	0.90

* Concentration of 100 p.p.m. N.
† Concentration of 0.004 per cent.
‡ Concentration of 1 gm./liter.

The oxygen consumption and respiratory quotient of *Rh. trifolii* in the different media are shown in table 2.

The respiratory quotients of these cultures were slightly different in some cases from the quotients given in table 1, but the same general changes with time were observed. The somewhat greater variability in respiratory quotient values in this experiment, as shown in table 2, in comparison with the quotients shown in table 1 can be largely accounted for by reason of the lower rate of

respiration of the cultures from which the data of table 2 were obtained. The influence of the *Az. vinelandii* medium extract resembled that of the other accessory materials in that it brought about a distinct lowering of the respiratory quotient of the organisms for some time after inoculation.

Another experiment was conducted in which the organisms employed for the inoculation had been cultured for several transfers in NH_4Cl-cane sugar medium. The influence of the various non-nitrogenous factors upon the

TABLE 3

Oxygen consumption and respiratory quotients of Rh. trifolii during consecutive hourly intervals in media containing different substances

Organisms for inoculum cultured in NH_4Cl medium

NH_4Cl* + SUCROSE C.P.						NH_4Cl* + BROWN CANE SUGAR		YEAST EXTRACT* + SUCROSE C.P.	
Control		Cysteine†		Soil extract‡					
O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.	O_2	R.Q.
cu. mm.		*cu. mm*		*cu. mm.*		*cu. mm.*		*cu. mm.*	
10.0	1.08	9.8	0.78	17.7	1.00	13.2	0.84	19.8	0.88
10.0	1.10	10.2	0.89	22.3	1.05	17.5	0.91	21.4	0.97
11.4	1.26	11.5	0.91	28.0	1.18	20.4	1.07	29.9	0.98
11.2	1.34	11.3	0.89	33.2	1.16	24.9	1.08	33.2	0.97
13.4	1.28	14.8	1.06	39.6	1.14	32.8	1.10	34.5	0.95
15.7	1.28	15.9	1.17	45.7	1.19	39.9	1.21	45.0	0.99
17.5	1.28	22.8	1.13	48.0	1.25	54.0	1.22	52.6	1.03
22.8	1.18	29.8	1.13	60.4	1.21	74.6	1.04	58.4	1.03
28.3	1.18	39.4	1.20	73.1	1.20	103.0	1.08	62.0	1.03
33.4	1.15	51.5	1.22	78.0	1.15	114.6	1.13	62.0	1.01
34.2	1.21	59.9	1.20	80.4	1.11	148.8	1.18	71.6	1.00
37.6	1.26	77.3	1.20	96.1	1.14	169.0	1.17	75.9	0.96
44.5	1.20	102.0	1.18	110.0	1.13	187.0	1.17	77.0	0.90
40.7	1.22	102.0	1.19	118.8	1.09	185.0	1.17	75.2	0.91
330.7	1.21	558.2	1.16	851.3	1.15	1,184.7	1.14	718.5	0.97

* Concentration of 100 p.p.m. N.
† Concentration of 0.002 per cent.
‡ Concentration of 1 gm./liter.

respiration of *Rh. trifolii* was tested with NH_4Cl as the nitrogen source. The oxygen consumption and respiratory quotients for the organisms growing in the different media are given for 14 consecutive hourly intervals in table 3.

The data of table 3 show changes in the respiratory quotients of the various cultures which were similar to those observed in the previous test. The initial reduction of the quotients was, in general, not so large as for the cultures reported in tables 1 and 2 where KNO_3 was employed as the nitrogen source.

The data in tables 1, 2, and 3 show the stimulative effect of the different

extracts upon oxygen consumption by *Rh. trifolii*. One difference between the data in the three tables is that the organisms which had been cultured in yeast extract medium previous to inoculation responded to the addition of cysteine to a much greater extent than did the organisms which had been growing in either the KNO_3 or NH_4Cl media. This might indicate that the organisms grown in the KNO_3 and NH_4Cl media had become somewhat adapted to a higher potential than those grown in yeast extract medium. On the other hand, the stimulation might be explained by the fact that the organisms grown in the yeast extract medium are able to carry appreciable quantities of accessory growth substances within their cells. This would enable them to respond to a greater extent to an improved potential of the medium than would be possible for organisms which had been largely freed from such factors. It seems probable that the differences in response to cysteine might be a result of a combination of the two causes suggested.

The data reported here entirely agree in that each factor which induced an increase in the rate of oxygen consumption by *Rh. trifolii* also brought about an initial decrease in the values of the respiratory quotients. It is not claimed that the reducing action of these substances is the complete explanation of their beneficial influence upon the rhizobia, but this effect does seem to be at least one of their important functions.

EFFECT OF AGAR UPON RESPIRATORY QUOTIENTS

The influence of the various stimulative extracts upon the nodule bacteria seemed to be related to some of the effects noted for agar. Allison and Hoover (1) found a higher growth rate of the rhizobia in KNO_3-sucrose c.p. medium when agar was present. They suggested that the stimulation brought about by agar was due to accessory growth substances present in the agar. Allyn and Baldwin (4) observed that agar facilitated the growth of the rhizobia in a KNO_3-mannitol medium. They also found that agar reduced the oxidation-reduction potential of the medium as much as 100 millivolts. From their studies it was concluded that the beneficial effects of agar upon the organisms could be satisfactorily explained by the change brought about in the potential of the medium. Although the studies of these different workers are not directly comparable, the differences in the explanations offered for the stimulative effects of agar seemed to merit further study.

A study was made, therefore, of the influence of agar upon oxygen consumption and the respiratory quotient of *Rh. meliloti*. Sodium nitrate and yeast extract were used as sources of nitrogen in concentrations of 100 p.p.m. of nitrogen. Glucose was the sugar used. Agar was added to the media at the rate of 15 gm. per liter. Two cubic centimeters of medium were placed in each manometer vessel. The vessels were allowed to stand long enough for the agar medium to solidify before inoculations were made. To each manometer flask, containing 2 cc. of medium, there was added 0.2 cc. of a suspension of *Rh. meliloti* which had been cultured free of accessory growth substances.

In the first experiment with agar, $NaNO_3$ (100 p.p.m. of nitrogen) was used as the nitrogen source in the medium. Both oxygen consumption and CO_2 production were determined for the various cultures. The oxygen consumption of the duplicate cultures was plotted against time. The resulting curves are given in figure 1.

The data in figure 1 indicate that in the absence of glucose the organisms were able to utilize only a very small fraction of the agar. In the presence of glucose, agar brought about a considerable increase in the rate of oxygen utilization. When yeast extract was substituted for $NaNO_3$ in a similar set of experiments no such stimulative effects were noted from the agar. It seems evident, therefore, that agar did not improve the yeast extract medium with respect to oxygen utilization by the organisms and that agar produced no beneficial effects upon *Rh. meliloti* that were not also produced by yeast extract.

In the presence of yeast extract, agar brought about no appreciable change in the respiratory quotients of the organisms. The bacteria growing on the agar-nitrate medium, however, had respiratory quotients quite different from those in the nitrate medium without agar. The respiratory quotients obtained in the same experiment as the data from which the curves in figure 1 were taken were plotted against time. The resulting curves are shown in figure 2.

Although the cultures in the presence of agar had a higher respiratory quotient for the first 2-hour interval the quotients decreased rapidly and continuously as growth continued. It seems probable that the organisms first utilized the liquid medium which separates from agar as it cools and so were not influenced appreciably by the agar during the first 2-hour interval. The lowering of the quotients as the growth of the organisms continued might be interpreted as resulting from the organisms' coming into more direct contact with the agar as the nutrients in the liquid medium were exhausted. Such a lowering of the respiratory quotients by agar is in harmony with the observation of Allyn and Baldwin that it brings about a lower potential of the medium.

The study of agar seems to indicate that the beneficial effect it exerts upon the rhizobia can be accounted for to only a very minor extent by the nutrient materials which it contains. Since it has been shown that agar reduces the oxidation-reduction potential of a nitrate medium and the present investigation indicates that it brings about a significant decrease in the respiratory quotients of the organisms, it seems probable that the reducing effect of the agar is, at least, one of the important reasons for the stimulative effect it exerts upon the rhizobia.

Attempts were made to measure the oxidation-reduction potential of different media under aerobic conditions with an electron tube potentiometer, but the results obtained were not very satisfactory. Indications were obtained, however, that the substances which stimulated the growth and oxygen utilization of the rhizobia and which lowered the respiratory quotient of the organisms also reduced the oxidation-reduction potential of KNO_3-sucrose c.p. media.

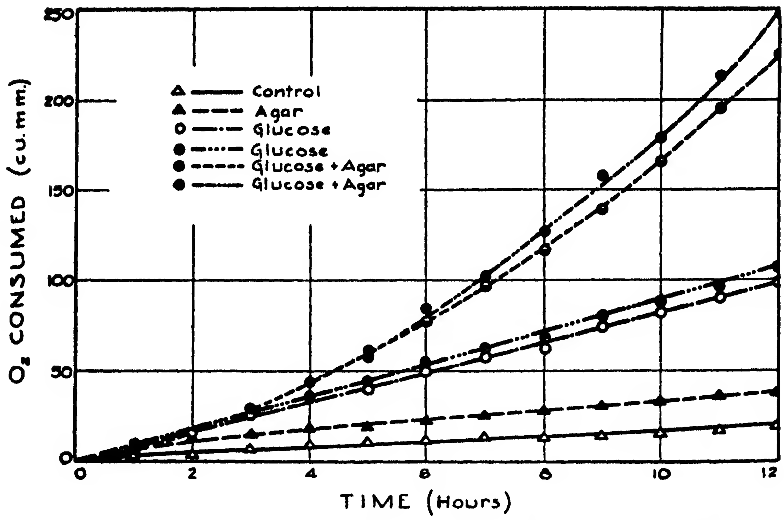

FIG. 1. OXYGEN CONSUMED BY RHIZOBIUM MELILOTI IN A NITRATE-MEDIUM WITH AND WITHOUT GLUCOSE AND AGAR PRESENT

Inoculum freed of accessory growth substances by culturing in KNO_3-cane sugar medium

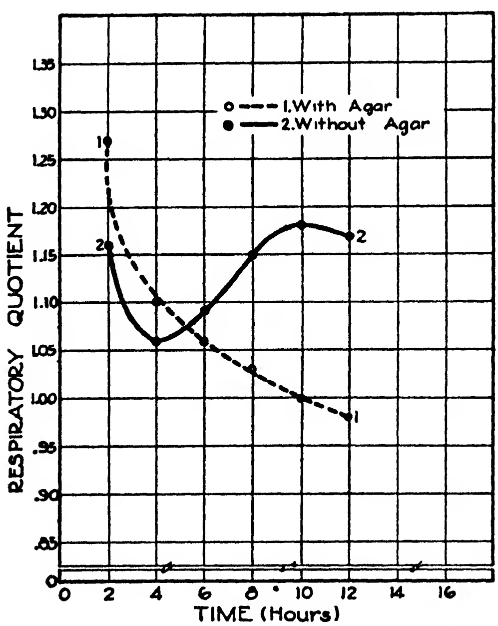

FIG. 2. THE RESPIRATORY QUOTIENTS OF RHIZOBIUM MELILOTI FOR CONSECUTIVE 2-HOUR INTERVALS WHILE GROWING IN A GLUCOSE-NITRATE MEDIUM WITH AND WITHOUT AGAR PRESENT

Inoculum freed of accessory growth substances by culturing in KNO_3-cane sugar medium

DISCUSSION

In a previous investigation concerning accessory growth factors for the rhizobia (7) a similarity was found between the stimulative effects of such materials as alcoholic extracts of cane sugar and brown cane sugar and the effects of reducing agents such as cysteine and thioglycollic acid. The data presented in the present paper show that the substances which were able to stimulate the growth and respiration of the rhizobia also brought about a decrease in the respiratory quotients of the organisms. The period of time during which the respiratory quotients of any culture was depressed varied with the different substances employed. Soil extract, *Az. vinelandii* medium extract, brown cane sugar, and cysteine, usually brought about a decrease in the quotients for only the first few hours subsequent to inoculation. With such materials as yeast and legume extracts the respiratory quotients were depressed over a much longer period of time. The important effect appeared to be the initial lowering of the quotient. Thus, yeast extract and brown cane sugar, in many cases, exerted about equal stimulative effects upon the oxygen consumption of the organisms. Brown cane sugar brought about a decrease in the respiratory quotient for only a very few hours, however, whereas yeast extract had a similar effect throughout most of the growth period of the organisms. These results seem to warrant the conclusion that the growth of the rhizobia in a new medium is greatly favored by the presence of an initial hydrogen donator or a reducing agent. The work of Allyn and Baldwin (3, 4) indicates that the function of such materials might be either to establish, or to aid the organisms in bringing about, a potential of the medium favorable to their growth.

The fact that cysteine was not able to induce as rapid growth and oxygen utilization of the organisms in the media composed of purified materials as were many of the other substances studied, suggests that the beneficial effects of these substances might not be restricted to the creation of a more favorable potential in the medium. It is not improbable, however, that the reducing effects of such materials as soil extract and brown cane sugar can not be simulated by such a drastic reducing agent as cysteine, even in very low concentrations.

It seems likely that the lowering of the respiratory quotients was a result of a direct physiological effect upon the organisms. The effect is most readily explained by the hypothesis that the various materials which brought about the lowering of the quotients acted as hydrogen donators to the organisms. Such substances as soil extract and brown cane sugar, which affected the respiratory quotients for only short periods of time, therefore, might be designated as "initial hydrogen donators." It appears probable that the presence of readily available initial hydrogen donators in the media would benefit the organisms, not only by enabling them to lower the potential of the medium to a more favorable point, but also by furnishing them a very available initial source of energy. This would enable the organisms to make the various adjustments

which seem to be necessary when bacteria are inoculated into a new medium. Such a theory is supported by the observation that the various materials, though furnishing almost negligible additions to the total energy supply of the media, brought about considerable decreases in the lengths of the lag periods of the organisms.

SUMMARY

The physiological effects of various materials upon *Rh. trifolii* and *Rh. meliloti* were studied by the measurement of oxygen consumption and CO_2 production in Warburg manometers.

Brown cane sugar, soil extract, *Az. vinelandii* medium extract, and cysteine added to KNO_3 or NH_4Cl media brought about a decrease in the respiratory quotient of *Rh. trifolii* for the first few hours after inoculation.

Yeast and alfalfa extracts lowered the respiratory quotient of *Rh. trifolii* throughout most of the period of active growth.

Agar brought about a lowering of the respiratory quotient of *Rh. meliloti* in a glucose-nitrate medium but not in a glucose-yeast extract medium.

Attempts to measure the oxidation-reduction potential of different media with an electron tube potentiometer were not very satisfactory. Indications were obtained, however, that the accessory growth substances studied reduce the oxidation-reduction potential of KNO_3-sucrose c.p. media.

One of the important functions of accessory growth substances for Rhizobium seems to be to provide an initial hydrogen donator. The rôle of a hydrogen donator for the nodule bacteria appears to be at least two-fold: first, it tends to lower the oxidation-reduction potential of the medium, and, second, it furnishes the organisms with a readily available initial source of energy which enables them to make the necessary adjustments for the establishment of favorable growth conditions.

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BOOK REVIEWS

Wild Flowers. By HOMER D. HOUSE. The Macmillan Company, New York, 1935. Second Printing. Pp. 362, figs. 35, full color illustrations 264.

The purpose of the author in preparing this attractive volume is indicated as follows: "The present volume is offered, with its wealth of color and form, scarcely approaching the beauty of the growing and living plants depicted, with the hope that the interest which it may stimulate in our native and naturalized wild flowers will become a potent force for their preservation and protection."

The work is particularly timely because of the rapidly growing interest among thinking people in our forests, prairies, parks, and arboreta. It is a part of the great conservation movement to which the Nation is committed. The author has rendered a distinct service to the large public interest in the conservation of wild life.

The book is well arranged and beautifully illustrated in color. It will bring much profit and pleasure to the student and to the general reader and should find a place on the shelves of our libraries.

Ferns of the Vicinity of New York. By JOHN KUNKEL SMALL. The Science Press, Lancaster, Pa., 1935. Pp. 285, map 1, illus. 85.

Lovers of this important group of our plant kingdom will find much satisfaction and pleasure in reading this book. The contents of the volume are best indicated by the following list of topics: Fern family—Polypodiaceae; Curly-grass family—Schizaeaceae; Cinnamon-fern family—Osmundaceae; Adder's-tongue family—Ophioglossaceae; Pepperwort family—Marsileaceae; Salvinia family—Salviniaceae; Horsetail family—Equisetaceae; Clubmoss family—Lycopodiaceae; Spikemoss family—Selaginellaceae; Quillwort family—Isoetaceae. Aside from these, the treatise contains a preface, introduction, map, taxonomic list with citations, comparative lists, authorities cited in this work, glossary, and index.

The author notes that "The amateur botanists of Manhattan and vicinity were the first on the Atlantic seaboard to organize themselves into an association—The Torrey Botanical Club—for the study of plants from a botanical standpoint, growing naturally within a certain region, in this case an area two hundred miles in diameter with Manhattan Island at the center was designated. This region has come to be known as the Local Flora Area." The author notes further that "The preparation of this work was prompted by the lack of a book devoted to the ferns of the Local Flora Area. It is based mainly on collections of ferns preserved in the herbarium of The New York Botanical Garden, besides studies in the field, both within and without the Local Flora Area."

The value of the book is materially increased by the numerous fine illustrations.

The Mushroom Handbook. By LOUIS C. C. KRIEGER. The Macmillan Company, New York, 1936. Pp. xiv + 538, figs. 126, colored plates 32. Price $3.50.

Commercial mushroom growing has already attained a rather definite place in our agricultural economy. The present work, with its beautiful illustrations and clear descriptions, is a distinct contribution to the subject. To quote the author: "In recent years considerable interest in mushrooms has become manifest. Even clubs for their study, so-called mycological clubs, have been formed in various parts of the country, particularly in the large cities. The Federal and most State governments have issued literature advising which kinds to eat and which to avoid. New York State may be said to have done not only pioneer but effective work in the accumulation and dissemination of knowledge concerning mushrooms." The author says, further: "It is not surprising that the public is interested in mushrooms. Several reasons exist. First and foremost, many are good to eat, as everyone knows who consults the bills-of-fare of the better restaurants. Some people, especially our Italian and Bohemian fellow-citizens, search woods and meadows for the spontaneous crop that is to be had for little more than the effort required in the picking. True, there are poisonous, even deadly kinds, but these one can learn to avoid by acquiring a little knowledge such as this book is intended to convey."

Aside from the list of illustrations, introduction and acknowledgments, and abbreviations, the topics dealt with are as follows: Field Study of Mushrooms and Other Fungi; Conditions Under Which Mushrooms Grow and Thrive; Forest Types, with Reference to Distribution of the Higher Fungi; How to Collect, Study, and Prepare Mushrooms for the Herbarium; Life History and General Characteristics of Mushrooms; Economic Importance of Fungi; Common Edible Mushrooms; Growing Mushrooms; Poisonous Mushrooms; The Wood-Destroying Fungi; The Literature on Mushrooms and Their Allies; and Systematic Account of Selected Larger Fungi. Names of the principal authors of fungus species; a bibliography, a glossary of technical terms, and an index are also given.

The mushroom grower and the general reader will readily acknowledge a debt to the author for having produced an interesting and readable book.

Soils—Their Origin, Constitution and Classification. An Introduction to Pedology. Second Edition. By GILBERT WOODING ROBINSON. Thomas Murby & Co., London, 1936. Pp. xvii + 442, figs. 17, plates 5, tables 44.

This is the revised issue of the first edition, which appeared in 1932. As an admirer of the late Dr. Marbut, the author inscribed the book "In Honour of Curtis Fletcher Marbut."

In the preface to the second edition, the author says:

In presenting the second edition of "Soils," I feel that some indication should be given of the changes which have been made from the original edition, and of the principles which I have followed in making such changes.

In the first place, I have made many small variations from the first edition by correcting erroneous or misleading statements and by clarifying obscure explanations. It is, perhaps, too much to hope that those parts of the first edition which survive have been completely purged of faults. Secondly, I have added some material which, although not modifying the original account, may augment its "corroborative detail." Here it has been necessary to exercise a certain economy in view of the abundance of material available. Thirdly, I have re-cast those portions of the book which appeared to require amendment in view of recent advances. These changes are mainly in the chapters on the Pedogenic Processes, on the Clay Complex, on Base Exchange, on Soil Moisture and on Soil Classification. Fourthly, I have added additional examples in the chapters on the Soil Groups and have somewhat amplified the chapter on Soil Geography. Lastly, I have withdrawn the appendix on Methods of Analysis in view of the publication of Mr. C. H. Wright's book on the subject. The narrative has been increased by 63 pages. When allowance is made for excisions and replacements, it will be seen that the amount of fresh material is considerable. About 150 new bibliographical references have been added.

The contents of the book include: preface to first edition; preface to second edition; contents; list of illustrations; and nineteen chapters designated as follows: Introductory; General View of the Constitution of the Soil; The Pedogenic Processes; The Clay Complex; Base Exchange and other Reactions of the Colloidal Complex; Soil Organic Matter; General Physical Properties of Soils; Water Relationships of Soils; Soils of the Podsolic Group; Tshernosems and their Related Groups; Ground-water Soils—Including Peats; Saline, Alkaline, and Soloti Soils; Soils of the Humid Tropics and Sub-tropics; Soils Associated with Calcareous Parent Materials; The Classification of Soils; The Geography of Soils; Soil Surveys; Soil Analysis; and Soils, Plant Growth, and Agriculture. In addition to these, there are an index of places and a subject index. The subject matter is well arranged and effectively presented.

Pedology. By JACOB S. JOFFE. Rutgers University Press, New Brunswick, New Jersey, 1936. Pp. xvi + 575, plates 41, figs. 4, tables 136.

A satisfactory definition of the term *pedology* is supplied by the author in his foreword:

Pedology is the science of soils. Its scope is the study of the phenomena presented to us by the soil body in its natural position. Pedology is thus that realm of natural science which has as its objective the elucidation of the natural laws governing the origin, formation, and distribution of soils. Pedology should not be confused with soil science, which is a broader subject, embracing pedology and its applied phases such as agronomy, soil fertility, soil reclamation, soil physics, soil chemistry, and soil biology or rather the physics, chemistry, and biology of soils. Although pedology is not a fundamental science as is physics, chemistry, or mathematics, it is an independent science dealing with a natural body, the soil, just as botany is an independent science dealing with a natural body, the plant, or zoology dealing with a natural body, the animal.

The treatment as developed by the author rests on the broad generalization laid down by the Russian School of Soil Investigators. The late Curtis F.

Marbut, of the United States Department of Agriculture, offers a clear explanation of the significance of the contributions made by the Russian School of Pedologists. In his introduction to Dr. Joffe's book he says:

At the time when western Europe was still engaged in the futile assertion that the soil as soil is dominated in its general features by the materials out of which it has been built, the Russian workers had already shown that the soil is the product of process rather than of material and is, therefore, a developing body rather than a static body. They allied the soil to life rather than to death.

Fortunate in the mid-continental location of the country in which they worked, equally fortunate in its broad geographic extent, the relatively slight extent to which man had interfered with the normal course of Nature's processes and the clearness with which the results of her processes in soil development are expressed, the Russian workers were able to see, with remarkable clearness, relationships that in western Europe are either obscurely expressed or not expressed at all.

In the clearness and simplicity with which the great soil features, as well as their relation to environment, are expressed, Russia occupies a place in the development of pedology comparable to that of the western United States in the development of the science of land form development. Whether our Russian colleagues are to be praised or merely congratulated may be a debatable question, but that the fundamental principles of the science of the soil were first laid down by them on the basis of conditions and facts in their own tremendously varied country is not debatable. Dokuchaev and his co-workers occupy the same position in pedology as do Sir Charles Lyell and his co-workers in geology, and Linnaeus in botany.

Aside from the frontispiece, the foreword by the author, and the introduction by Dr. Marbut, the book is made up of two parts—Soil Genesis and Soil Systematics—, and appendices. Part I contains six chapters, entitled, respectively: Pedology as a Scientific Discipline; Soil as a Natural Body; Soil Defined, Soil Morphology and Methods of Studying it; Soil Genesis: Weathering and Soil Formation; Soil Genesis: Soil Formers; and Soil Genesis: Soil Forming Processes. Part II contains ten chapters which are entitled, respectively: Desert, Semi-Desert, and Arid Steppe Types of Soil Formation: Gray, Brown, and Chestnut Brown Soil Zones; Chernozem Type of Soil Formation; Podzol Type of Soil Formation; Subtypes and Transition Types of Soils in the Podzol Zone; Tundra Type of Soil Formation; Laterites and Lateritic Type of Soil Formation; Intrazonal Soils: Solonchak, Solonetz, Solodi, Rendzina, etc.; Bog and Marsh Soils; Mountain Soils; and Some Pedological Features of the Soils of the United States. A selected list of books and monographs on soils with special reference to pedology is also supplied. The index of authors and also the index of subjects are helpfully arranged.

The present volume will render a wide and useful service, particularly in view of the growing interest in soil conservation, the relation of soil environment to land use, and the state, regional, and national policies centering around methods of soil management and guided by the recent as well as older information that has been gained by soil technologists in this and other countries.

Les Sols de France au Point de Vue Pédologique. By V. AGAFONOFF with introductory statements by M. A. LACROIX, M. L. LUTAUD, AND M. A. DEMOLON. Dunod, Paris, 1936. Pp. xiii + 154, maps 2, tables 23, figs. 23.

The author obtained his early training and experience in Russia. He has prepared his treatise on the basis of the methods of soil classification developed by the Russian pedologists. The work is an important contribution to the mapping and classifications of the soils of France. The importance attached to the work by Dr. Agafonoff's colleagues is indicated in their introductory statements. The following quotations are, in the order given, from the statements of Lacroix, Lutaud, and Demolon.

The scientific study of the soil is his passion. Familiar with the methods of the Russian School of Pedologists, he has made it his task, first of all, to make these familiar and appreciated in France—not under the form of a theoretical exposition, but by employing a more personal procedure, which consists in undertaking a study of our own soils from a point of view quite different from what has been considered until the present time.

...... For the first time the methods and results of the Russian School of Pedologists have been applied to our own country by a prominent investigator, who has not hesitated during the last few years to undertake a systematic study of our different provinces, to observe the soil directly, to collect personally, with methodical care, samples necessary for this study. This work appears now as the result of personal, patient and careful efforts. This is a new book for us and, what is more important, it opens a new point of view. All those who read it will recognize the interest and talent with which the author has conducted and achieved his task.

...... With admirable perseverence, M. V. Agafonoff has applied during many years to the soils of France the principles of those that laid the foundation of pedology—Dokoutchaïev, Sibirtzev and Glinka. The importance of climate and vegetation in the origin and evolution of soils represents a great idea, which has taken the place of the previous insufficiently comprehensive conceptions of the true nature of the soil. In this new conception biology now plays a very important part. In a parallel manner, the methods of examination of the soil were modified and the consideration of the profile enlarged the field of exploration. which was too limited at the surface. It is not exaggeration to say that a study of the soil has brought about during recent years a complete rejuvenation and a new orientation. We are indebted to M. V. Agafonoff, who has contributed largely, by his pedological studies, to the soils of France.

The four chapters of the book are entitled, respectively: Pedology—A New Science; The Research Plan and Our Methods for the Examination of the Soils of France; Soil Regions of France; and Azonal Soils.

Soil Science—Its Principles and Practice. By WILBERT W. WEIR. J. B. Lippincott Company, Chicago—Philadelphia, 1936. Pp. vii + 615, figs. 134.

The title "Soil Science" has apparently not been used previously for the designation of textbooks dealing with soils, soil fertility, and fertilizers. The term has been used for the designation of the international journal SOIL SCIENCE, established by the reviewer in 1915. It was the author's intention to present the subject in its broad relations, even though he has stressed the applied phases of soil science.

The opening paragraph in the author's preface reads as follows:

Possibly there is no subject that lies closer to the welfare of the human race, with its population of nineteen hundred millions of people, than the production and maintenance of

the necessary food supply; and it is particularly gratifying to know that today soil science has so far advanced that one may confidently assert that, in the absence of some catastrophe of nature or a war of extinction, mankind need never fear starvation or the want of food. Such an eventuality has been made unlikely by scientific methods of agriculture, increased knowledge of the soils themselves, better understanding of plant growth, and improved methods for taking care of and preserving the soils.

A further examination of the contents of the book shows it to embrace 27 chapters and an index. The several chapters are designated as follows: Development of Agriculture and Rise of Scientific Thought; Physical Constitution of Soils; Physical Properties of Soils; Chemical Nature of Soils; Microbial Population of Soils; The Modern Concept of Soils; Soil Classification; Natural Order in Soils; Soil and Plant Relationships; Crop Production and Soil Fertility; Tilth and Tillage; Soil Water and Soil Fertility; Irrigation and Land Drainage; Aëration as a Factor in Soil Fertility; Soil Reaction and Soil Fertility; Soil Acidity and Soil Alkalinity; Science and Art of Liming Acid Soils; Organic Matter and Soil Fertility; Micro-organisms and Soil Fertility; Plant Nutrient Elements; Fertilizers; Fertilizers, Their Effects on Soils and Plants; Fertilizing Plants to Meet Their Nutrient Requirements; Crop Rotation and Soil Fertility; Soil Erosion; Determining the Need for Fertilizers; and Interpreting Results of Fertilizer Experiments.

The reader's attention may be directed particularly to the fact that the author has made himself thoroughly familiar with soil research at the American agricultural experiment stations and has presented some of their important findings in a readable and logical form.

Problems in Soil Microbiology. By D. W. CUTLER AND L. M. CRUMP. Longmans, Green and Co., London—New York—Toronto, 1935. Pp. vii + 104, figs. 18, tables 32, maps 1. Price $3.20.

The authors note in the preface:

This book incorporates the substance of a series of lectures delivered by one of us (D. W. C.) at the University College of Wales, Aberystwyth, on the Aberystwyth Lecture Foundation during the session 1934–35. The contents are largely the result of work carried out in the General Microbiology Department of the Rothamsted Experimental Station by the authors and their colleagues, and it is in no sense a textbook of soil microbiology.

The theme throughout is to show that from the biologist's viewpoint the soil is an eminently suitable home for living organisms and that, through the long ages of evolution, a population has been selected which is, on the whole, so unspecialized that almost any substance which finds its way into the soil, either naturally or in the course of modern agricultural practice, will eventually become incorporated into the general soil economy.

The contents of this interesting book are indicated in the titles of the seven chapters, which are as follows: The Suitability of the Soil for Micro-organisms; The Bacterial Population under Field Conditions; The Relation of Bacteria to Nitrite; Carbon Dioxide Production by Soil; The Growth of Protozoa in Pure Culture; The Behaviour of Protozoa in Soil; and The Inter-Actions between

the Soil Organisms. The authors have also provided a bibliography, an index, and a map of protozoan distribution.

The History of the Upper Mississippi River in Late Wisconsin and Postglacial Time. By WILLIAM S. COOPER. The University of Minnesota Press, Minneapolis, Minn., 1935. Pp. xii + 116, figs. 46, plates 4. Price $4.00.

The nature of this treatise is indicated in the author's foreword. He says:

> This study in glacial geology is the work of a botanist who is a firm believer in what has been aptly termed "cross-fertilization of the sciences." It developed gradually from a beginning that was purely botanical. An area of ancient dunes close to Minneapolis supports a flora that is distinctive and of unusual interest. An ecological investigation begun here necessitated consideration of the dunes themselves. Steadily the field broadened in the manner familiar to every scientific worker. The dunes led back to their source in the outwash. A great glacial lake disclosed itself. Finally the various elements resolved themselves into a history, in which the Mississippi River plays the leading rôle. Concurrently the botanical field expanded until it covered the vegetation of both dunes and outwash, with particular emphasis upon its development during postglacial time. The foundational geologic study is here presented, the botanical part is still unwritten.

The contents of the treatise consist of: The Field of Inquiry; The Patrician (Middle Wisconsin) Glaciation; The Late Wisconsin Glaciation; Postglacial Entrenchment of the Mississippi and St. Croix Rivers; The Dunes; a bibliography; and an index.

The author has succeeded in telling an interesting story—interesting both as to facts and implications. One senses in reading the book the part that has been played by The Father of Waters in carving the face of North America.

A Provisional Soil Map of East Africa (Kenya, Uganda, Tanganyika, and Zanzibar) with explanatory Memoir. By G. MILNE. East African Agricultural Research Station, Amani—Tanganyika Territory, 1936. Pp. 34. Price 5/— post free.

The soil technologist may, with propriety, apply a trite saying to his field of study—"the world is growing smaller" in respect to soil classification and mapping. Much has been added within the past five years to our inventory of the soil resources of the world. Notable contributions were made in this field by the Third International Congress of Soil Science, which was held at Oxford, England, in 1935. Some of the data appearing in the present memoir were presented at that Congress. The students of soils will note that the map in question includes Kenya, Uganda, Tanganyika, and Zanzibar. Thus, we are beginning to know more about the soil resources of a continent where human civilization saw its early beginnings.

The memoir is made up of two parts entitled General Description, and the Soils of the Four Dependencies. The specific topics dealt with in these parts and the names of the contributors to the second part are given below: Part I: General Description—Aims, Origin, and Authorship; Area Included, Outline,

and Scale; Sources of Information; Classification of Soil Types,—Table of Soil Groups, Desert Soils, Saline Soils, Plains Soils, Black or Grey Clays, Mottled Clays, Red Earths (two groups), Plateau Soils, Podsolised Soils, and Lithological Types; Colouring and Notation; Occurrence of Soil Types in Association,—The Catena, Other Complex; and General Conclusions. Part II: The Soils of the Four Dependencies: Kenya Colony and Protectorate (V. A. Beckley and G. H. Gethin Jones); The Uganda Protectorate (W. S. Martin and G. Griffith); Tanganyika Territory (G. Milne); and the Zanzibar Protectorate (L. W. Raymond).

The soils described in this volume are interesting from many points of view. The authors have done pioneering work and have carried on their tasks even under trying and difficult circumstances. Their contribution gains in value because of the circumstances under which their work was performed.

Die Lage der Landwirtschaft im Freistaat Sachsen—Heft 3. By WOLFGANG WILMANNS AND HERMANN ISENSEE. Verlag von Theodor Steinkopff, 1936. Pp. vi + 72, illus. 20, maps 2. Price 2.50 RM. (paper cover).

The investigation of agricultural production is one of the major issues of present-day Germany. Hence the description of the status of agriculture in Saxony as given in the present volume should interest agronomists and agricultural economists. The report is made up of two parts dealing respectively with the agricultural conditions in Saxony and the status of production and its effect on the agricultural conditions in the harvest years 1931–1932 and 1932–1933. The general topics discussed in Part I are the General Basis of Agriculture in Saxony and the Status of Progress in Agriculture in the harvest years 1931–1932 and 1932–1933. Part II contains as its major topics General; Methods of Study; and Results. Altogether the report contains much interesting material and would furnish helpful information to the student of European agriculture.

Fertilizer in Crop Rotations—Issue I—A Collection of Papers edited by S. P. KULZHINSKII, A. S. CHERNAVIN, AND S. S. SIGARKIN. Moscow and Leningrad, U. S. S. R., 1934. Pp. 216.

The major topics dealt with in the book are as follows: Under Problem of Studying and Working out a Fertilizer System in Crop Rotations, by S. S. Sigarkin; Agro Technical Principles of Working out a System of Fertilization, by I. V. Yakushkin; Legumes in Crop Rotations as a Factor of the Effectiveness of Fertilizers, by S. P. Kulzhinskii; Manure in Crop Rotations, by B. G. Naidin; Green Manures in Crop Rotations, by E. K. Alekseev; Peat and Peat Fertilizers in Crop Rotations, by B. A. Kabanov; Liming in Crop Rotations, by S. S. Yarusov; Rock Phosphate Flour in Crop Rotations, by S. V. Shcherba; A System of Fertilization and a Study of it in Economies of 100 Percent Chemization Conducted by the Institute of Fertilizers and Agro-Pedology, by A. I. Tutkevich; Fertilizers in Crop Rotations with Flax, by Ya. V. Peive; Fertilizer

Placement in Crop Rotations with Grain, by I. V. Yakushkin; Fundamental Principles in Working Out a System of Fertilization in Sugar Beet Culture, by G. B. Neiman; On the Problem of the Systems of Fertilizers in Vegetable Gardening, by S. T. Antoshin.

The rapid development of agricultural research in the Soviet Union adds interest to the efforts now being made in that country to develop the rational use of agricultural land and the maintenance and improvement of yield levels with the aid of crop rotations and fertilization.

Ergebnisse Der Agrikulturchemie. Part IV, 1935. Edited by F. ALTEN AND M. TRÉNEL. . Verlag Chemie, Berlin, 1936. Pp. 229, 87 tables, 40 illus. Price 12 RM.

This is the fourth volume of a series. The present work is a desirable addition to the various reviews on progress in agricultural chemistry. The nature of the data dealt with by the author is clearly noted in the table of contents. Sections A to E are designated respectively: General; Investigation of Soils; Fertilization; Feeding; and Nutrition. A general review supplied by the authors may be noted as follows:

> With the publication of this volume the tradition is continued of annually collecting the important reports from the division of agricultural chemistry of the convention of the Union of German Chemists. The suggestions and scientific points of view given in the reports should reach other scientific groups through this publication. The reports give an excellent idea of the scientific status of agricultural chemistry. They show the important part played by the studies of the agricultural chemists in practical agriculture and at the same time give the scientist a perspective of new chemical problems in production.
>
> The "Ergebnisse der Agrikulturchemie" is not only a valuable source of scientific inspiration to the chemist but it is also a practical and indispensable aid to the agricultural advisor and teacher.

The authors and their collaborators have done well in creating a picture of progress in a very important field of agricultural science. Teachers, students, and progressive farmers should add this volume to their collection of reference material.

Colloids in Agriculture. By C. E. MARSHALL. Edward Arnold & Co., London, 1935. Pp. viii + 184, figs. 14, tables 12.

The field of colloidal chemistry has already furnished a vast quantity of data which serve to explain fundamental facts and relations in agricultural production and the utilization of agricultural products. To quote the author's own words: "I have written this book with an eye to the needs of two classes of readers. Firstly, I hope it will prove useful to our county organizers, district lecturers and rural instructors—indeed, to all who are engaged in scientific agriculture and who are interested in the application of our latest knowledge to their field work and their teaching. I have written also for our agricultural students. To them, few subjects are more confusing than colloid chemistry, due largely to our haphazard methods of teaching."

Part I of the book deals with the Realm of Colloids. Respectively, the chapters are designated: The Nature of Colloids; The Formation of Colloids; The Properties of Small Particles in Suspension; The Properties of Molecules at Surfaces; Ions at Surfaces; and Soils and Gels. Part II deals with Colloids in the Soil, and contains the following chapters: The Mineral Colloids of Soil; The Organic Colloids of Soil; Colloids in Soil Formation Processes; and Colloids and Soil Texture. Part III has to do with Colloids in Plant and Animal Life. It contains the following chapters: Some Colloidal Materials Present in Living Organisms; Colloidal Architecture in Biological Structures; Milk and Milk Products as Colloidal Systems; and Smoke Damage and Plant Protection. These are followed by an index.

This book will no doubt be accepted as a welcome addition to the reference shelves of teachers, students, and progressive farmers.

Die Methoden zur Bestimmung des Kali- und Phosphorsäure-Bedarfs landwirtschaftlich genutzter Böden. By WALTER-ULRICH BEHRENS. Verlag Chemie, G. M. B. H., Berlin, 1935. Pp. 196, tables 140.

There is much interest at the present time in the so-called rapid methods for the determination of soil deficiencies in respect to potassium, phosphorus, calcium, and other plant nutrients. The present work deals with the subject from the scientific as well as the technical point of view. As is noted by the author in his preface, "The number of contributions relating to the determination of the plantfood needs of our cultivated soils has become unusually large. I have gone through the material and have brought together the most important results." The author also notes that "A particular effort was made to carry out a critical review of the different methods." In this way, he has rendered a helpful service to many workers in the field.

The major divisions of the book deal with: A—Underlying Principles; B—The Individual Methods of Soil Investigation in Respect to the Requirements of Potash and Phosphoric Acid; and C—The Technique of Sampling and of Analysis. There are a bibliography of 13 pages and author and subject indexes.

Arbeiten über Kalidüngung. Vol. II. By O. ECKSTEIN. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, 1935. Pp. 478, illus. 123, tables 198.

Volume I of this treatise was reviewed in the June, 1932, issue of SOIL SCIENCE.

Summaries of the four major divisions of the book are given in English. The titles of these divisions are: Soil Research; Physiological Investigations; Investigations on the Influence of Commercial Fertilizers on the Chemical Composition and Nutritional Value of Food and Feeding Stuffs; and List of Papers Published by the Experiment Station, Berlin-Lichterfelde. There is also a subject index.

To quote from the preface in English as given by the author:

In nearly every country to-day there is a general opinion that agriculture can only be made to pay by the intensive use of all auxiliary methods which science can place at the disposal of the practical farmer. In countries where the area of cultivated land is relatively small, the realisation of the above point is shown by the fact that every possible acre is raised to the highest degree of productivity by the use of the best pedigree seeds or plants, thorough cultivation, careful regard to drainage and by the judicious and liberal use of commercial fertilisers. . . . As has already been pointed out, agricultural problems, and especially the handling of soil questions, are fundamentally dependent upon local agricultural conditions. It is of special interest in this respect to compare conditions in different countries, e.g. in the United States of America with those in Germany. The function of the German farmer is to secure as large yields as possible, consistent with suitable quality, and especially maximum yields of crops rich in protein, fat and fibers respectively. In the United States of America, however, where agricultural over production during recent years has been an economic calamity, the problem is quite a different one. In most oversea countries the problem is to raise the quality of the crops, so far as quality influences the capacity of the market to absorb them, to as high a degree as possible and to promote the culture only on the most suitable soils and by the best possible crop treatment.

There are a bibliography of 47 numbers and a subject index.

JACOB G. LIPMAN.

BASE EXCHANGE IN SOIL SEPARATES AND SOIL FRACTIONS (SAND AND SILT)[1]

ALFRED T. PERKINS AND H. H. KING

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Received for publication July 16, 1936

Base exchange phenomena in soils are the zeolitic-like abilities of soils to hold bases against water solution but permit their liberation by exchange with other positively charged ions.

The base exchange capacity of a soil is probably as good a laboratory test of the potential fertility of a soil as is now available. In general, soil chemists believe that the soil minerals possessing the power to exchange bases are chiefly the hydrated alumino silicate complexes and that the base exchange capacity of a soil increases as its colloidal content increases. Kelley and Dore (3) expressed this idea by stating that soil colloids are related to montmorillonite and that the replaceable base content may be increased several fold by grinding certain minerals.

The soils used in this investigation were Wabash and Summitt, which are relatively fertile, and Derby and Cherokee, which are relatively unfertile. The soils were treated with 6 per cent hydrogen peroxide to destroy all organic matter, and the residue was moistened sufficiently with water to form a thick mud and manipulated with the hands until all microscopically visible aggregates were broken down. Repeated treatments of this nature, as the separation progressed, were needed to insure the complete destruction of all microscopically visible aggregates. The soil was finally agitated in water at 25° in a large hydrometer jar and permitted to settle for $6\frac{2}{3}$ minutes for each 2 feet of depth. The supernatant suspension was syphoned off and the residue reagitated and allowed to resettle. This process was repeated until the supernatant liquid was apparently clear, after which the precipitates were collected. Other separations were made with sedimentation periods of 40 minutes, 4 hours, 24 hours, and 6 days. The entire scheme of separation and subsequent fractionation is outlined in table 1. The fractionations were made according to the specific gravity of the soil particles. The separates were suspended in a carbon tetrachloride-bromoform mixture, adjusted to the desired specific gravity, and centrifuged. The finer soil separates were moistened with carbon tetrachloride and placed in a high vacuum to remove all absorbed air, which would tend to reduce the apparent specific gravity of the particles.

[1] Contribution No. 210, department of chemistry. The data in this paper were presented at the Kansas City meetings of the American Chemical Society.

This laborious system of obtaining the soil separates was used in preference to an elutriating device, as it enabled one much more readily to watch the process of separation, facilitated the working of the soil to break down the aggregates, and made possible a more accurate separation of the particles at the desired points. An elutriating device will probably be used, however, in the future work.

The general trend of base exchange phenomena in the soils studied is indicated by the data given in table 2. It should be noted that such sedimentation periods were selected as would result in the various separates decreasing progressively by approximately 50 per cent in diameter. It is obvious that as the size of the particles becomes smaller the base exchange capacity of the separate increases per unit weight but decreases per unit of surface area. This agrees with work published by Perkins and King (4). According to the

TABLE 1

Soil separates and soil fractions

SOIL SEPARATES			SOIL FRACTIONS OBTAINED FROM SEPARATES		
Suspension time	Sedimentation time	Separate	Specific gravity		
			Minimum	Maximum	Fraction
....	6.67 min.	A	...	...	....
6.67 min.	40.00 min.	B	X	2.0	2.0–
40.00 min.	4.00 hr.	C	2.0	2.4	2.0–2.4
4.00 hr.	24.00 hr.	D	2.4	2.6	2.4–2.6
24.00 hr.	6 days	E	2.6	X	2.6–
6 days	....	F	...	...	....

A Wabash C 2.0–2.4 fraction is the Wabash soil minerals that will remain suspended in 2 feet of water at 25°C. for 40 minutes but will settle out in 4 hours and have a specific gravity between 2.0–2.4.

American System of mechanical analysis of soils the A separate would be sand, the B and C separates silts, and the D separate would be on the border line between silt and clay. According to the International System the A and B separates would be sands, the C and D separates silts, and the E separate would be on the border line between silt and clay.

The base exchange of the individual fractions of the soil separates was also studied. The data for the various fractions are presented in table 3. We note that in considering the A, B, C, and D separates the fractions with a specific gravity between 2.0 and 2.4 possessed a greater base exchange capacity than did the heavier fractions, but the difference decreased as the particles became smaller. In the A separates the fraction with a specific gravity between 2.0 and 2.4 possessed 41.5 per cent of the base exchange capacity of the entire separate even though it comprised only 9.6 per cent of the separate. As the particle size of the separates became smaller to the base exchange capacity

per unit weight became greater. In considering the 2.0–2.4 fractions alone, however, the base exchange capacity remained practically constant per unit

TABLE 2

Base exchange capacity of soil separates

(Average of Wabash, Cherokee, Derby, and Summitt soils)

SEPARATE	AVERAGE DIAMETER	BASE EXCHANGE CAPACITY PER 100 GM.	BASE EXCHANGE CAPACITY PER 100,000 SQ. CM. SURFACE*	SEPARATE, PER CENT OF ENTIRE SOIL	BASE EXCHANGE CAPACITY OF SEPARATE, PER CENT OF BASE EXCHANGE CAPACITY OF SOIL
	mm.	*m.e.*	*m.e.*		
entire soil	?	16.39	?	100	100
A	0.0560	6.36	15.23	28.2	11.61
B	0.0283	6.23	7.74	28.3	10.75
C	0.0111	15.42	7.52	13.1	11.54
D	0.0051	20.95	4.56	5.6	6.84
E	0.0025	21.86	1.98	3.7	5.20
F	?	46.69	?	21.1	54.06

* Calculated on assumption that particles are spheres with a specific gravity of 2.6.

TABLE 3

Base exchange capacity of soil fractions

(Average of Wabash, Cherokee, Derby, and Summitt soils)

SOIL FRACTION*	BASE EXCHANGE CAPACITY PER 100 GM	BASE EXCHANGE CAPACITY PER 100,000 SQ. CM SURFACE	FRACTION, PER CENT OF SEPARATE	FRACTION BASE EXCHANGE CAPACITY, PER CENT OF SEPARATE BASE EXCHANGE CAPACITY
	m.e	*m.e.*		
A 2.0–2.4	19.5	49.44	9.6	41.5
A 2.4–2.6	4.1	10.66	45.8	41.7
A 2.6–	1.7	4.42	44.7	16.8
B 2.0–2.4	20 1	23.72	9.4	34.6
B 2.4–2 6	5.3	6.25	43.3	42.0
B 2.6–	2.5	2.95	47.3	23.4
C 2.0–2 4	25.0	11.93	20.8	47.7
C 2.4–2.6	13.1	6.25	27.5	33.0
C 2.6–	4.1	1.96	51.5	19.3
D 2.0–2 4	20.5	4.51	43.8	57.3
D 2 4–2.6	12.7	2.79	45.5	36.9
D 2.6–	8.7	1.91	10.5	5.8

* The E and F separates were not fractionated. Because of their small size and tendency to absorb air etc., no satisfactory way to separate the individual particles according to specific gravity has been devised. A definite trend in the A, B, C, and D separates is evident.

weight regardless of the degree of fineness. The base exchange capacity of the 2.4–2.6 and 2.6–+ fractions increased as the degree of fineness increased. All

fractions showed decrease in base exchange capacity per unit area as the size of the particles decreased.

These data tend to indicate that there are at least two minerals present in the soil studied which are active in base exchange phenomena and that they differ somewhat in their base exchange characteristics.

Of the four soils studied the two fertile soils contained a much larger percentage of light minerals than did the unfertile soils. This is considered a separate problem, and additional soils are being investigated before publication.

Recently two articles have appeared in the literature dealing with the general problem presented in this paper. Hissink, Hooghoudt, and van der Spek (2) relate particle size of soils with soil properties, and Elder and McCall (1) associate specific gravity of soil particles with soil classification.

CONCLUSIONS

On a basis of the four soils studied the following points appear to have been established:

As particle size in soil separates decreases, base exchange capacity increases per unit weight but decreases per surface area.

As particle size in soil fractions decreases, base exchange capacity of the 2.0–2.4 fraction remains constant but that of the 2.4–2.6 and 2.6–+ fractions increases per unit weight.

All soil fractions as they become finer exhibit smaller base exchange capacity per unit surface area.

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MANGANESE IN NEW JERSEY SOILS[1]

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Years of careful study and investigation have led plant physiologists to the definite conclusion that manganese is one of the elements essential for plant growth. The literature on this subject is extensive and has been so fully reviewed that only a few references need be given here (2, 3, 4, 5, 8, 9). Careful experimental work has also shown that normal soils which are slightly to moderately acid usually contain sufficient manganese for normal plant growth. This manganese may or may not be in an available form, depending upon the soil reaction. For example, heavy liming may convert the soluble manganese into an insoluble or slowly soluble form, in which case there may be an actual manganese deficiency as far as the growing plant is concerned. This condition has been observed in the case of overlimed lettuce and spinach soils. Generally such a condition can be improved by applying a soluble manganese salt, as, for example, manganese sulfate, or by reducing the pH of the soil to a point where more manganese may be brought into solution. It rarely happens that manganese is present to such an extent as to prove toxic.

In view of the widespread interest in this subject, it has seemed worth while to make a survey of the manganese in the soils of New Jersey. To that end, samples of soil representing the most important types found in the state have been analyzed for manganese.[3]

In some cases a considerable number of soil samples have been collected from areas where the soils do not differ very much as to type, as for example Sassafras loams and sandy loams. In such cases it has seemed sufficient to report the average manganese content, with the maximum and minimum, rather than to report each individual sample. This plan has been followed with samples collected from the important potato areas of Middlesex and Monmouth Counties, largely loams and sandy loams; with similar samples collected from farms in the vicinity of Plainsboro where dairying is important and from a dairy farm at Juliustown; and with a few samples of loams and silt loams from dairy farms in Somerset County. In addition, manganese has been determined in samples

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[2] The authors are indebted to Mr. Patrick Martin, E. R. A. chemist, assigned to the soil department, for the major portion of the manganese determinations on the soil samples.

[3] For the determinations of manganese in soils from the Sussex area, New Jersey, see (1). Robinson also has determined manganese in some important American soils (6, 7).

TABLE 1

Manganese content of various soils

	MnO IN UNCULTIVATED SOILS	MnO IN CULTIVATED SOILS
	per cent	*per cent*
Loams and sandy loams from farms in the vicinity of Plainsboro. Average of 23 samples (maximum 0.084, minimum 0.019)	.....	0.055
Sandy loams from Juliustown. Average of 5 samples (maximum 0.019, minimum 0.013)	.....	0 016
Loams and sandy loams from potato areas of Middlesex County. Average of 19 farms	0.034	0.039
Loams and sandy loams from potato areas of Monmouth County. Average of 21 farms	0 035	0.035
Loams and silt loams from Somerset County. Average of 6 farms	0 095	0 093
Blueberry soils from Cranbury Substation, Burlington County:		
Average of plots A, B, C	.....	0.013
Average of plots H to N	.....	0.007
Virgin soil	.....	0 006

Belvidere Area

SOIL NUMBER		MnO IN CULTIVATED SOILS
		per cent
480	Washington loam	0.232
484	Chester gravelly loam	0 142
496	Dutchess loam	0 084
500	Dover loam	0.116

Bernardsville Area

640	Dunellen loam	0.116
650	Merrimac loam	0 131
658	Merrimac sandy loam	0.052
662	Gloucester loam	0 065
666	Clyde silt loam	0.013
678	Montalto loam	0 129
682	Croton silt loam	0.032
684	Whippany loam	0 032
704	Penn gravelly loam	0.258
718	Penn gravelly silt loam	0.194
728	Penn silt loam	0.071
714	Hagerstown silt loam	0.168
700	Chester loam	0 155
720	Washington loam	0.207

Camden Area

326	Sassafras loamy sand	0.019
338	Sassafras sandy loam	0.019

TABLE 1—*Concluded*

SOIL NUMBER		MnO IN CULTIVATED SOILS
	Camden Area—Concluded	
		per cent
360	Sassafras sand	0.019
397	Collington loam	0.039
416	Collington sandy loam	0.019
422	Collington sand	0.019
455	Keansburg loam	0.013
	Chatsworth Area	
735	Lakewood sand	0.013
749	Collington sand	0.019
751	Collington fine sandy loam	0.019
794	St. John's sand	0.019
	Freehold Area	
227	Sassafras loam	0.052
239	Sassafras sand	0.013
264	Sassafras fine sand	0 013
272	Sassafras fine sandy loam	0 019
284	Collington sand	0 013
315	Collington sandy loam	0.013
295	Colts Neck loam	0.039
233	Colts neck sandy loam	0 026
308	Keyport loam	0.019
	Millville Area	
636	Sassafras sand	0.013
526	Sassafras sandy loam	0.039
536	Lakewood sand	0.013
610	Norfolk sand	0.013

representing a number of soil types in six of the ten soil areas of the state. This gives a fair evaluation of the manganese content of the more important soil types found in the state. It is of interest to note that the soils of north Jersey, especially the loams, silt loams, and shale loams, generally contain a higher percentage of manganese than do the lighter soils of south Jersey. This is due in part at least to the manner of formation, the soils of south Jersey being predominantly coastal plain soils of marine origin, whereas those of north Jersey are predominantly residual, glaciated, and alluvial and therefore retain more of the manganese that was in the original rocks. The Lakewood, Collington, and Colts Neck sands generally show a low percentage of manganese.

Manganese has been determined in a large number of soils as indicated in table 1.

The manganese content of Penn shale loam which has been under definite fertilizer treatment for a period of years is reported in table 2. This soil is rather high in manganese, about twice as high as is Sassafras loam. If a very small fraction of this total could become available each year, it would undoubtedly supply the needs of most growing plants. Fertilizer and lime treatments do not appear to have had a definite influence on the percentage of manganese in the soil.

TABLE 2

Manganese in Penn shale loam—1932 samples

(Continuous fertilizer treatment since 1898)

CYLINDER NUMBER	FERTILIZER TREATMENT	MnO IN SOIL
		per cent
8A	Complete fertilizer, nitrogen as $NaNO_3$. Unlimed	0.129
8B	Complete fertilizer, nitrogen as $NaNO_3$. Limed	0.114
8C	Complete fertilizer, nitrogen as $NaNO_3$. Limed	0.103
17A	Complete fertilizer, nitrogen as $(NH_4)_2SO_4$. Unlimed	0.103
17B	Complete fertilizer, nitrogen as $(NH_4)_2SO_4$. Limed	0.119
17C	Complete fertilizer, nitrogen as $(NH_4)_2SO_4$. Limed	0 129

TABLE 3

Percentage of manganese (MnO) in horizon samples from field plots (1934 samples)*

HORIZON	UNLIMED SECTION							LIMED SECTION						
	Plot 2A	Plot 3A	Plot 7A	Plot 9A	Plot 10A	Plot 11A	Plot 18A	Plot 2B	Plot 3B	Plot 7B	Plot 9B	Plot 10B	Plot 11B	Plot 18B
A_p	0.065	0.064	0.039	0.039	0 019	0 026	0 026	0 023	0.019	0 019	0 019	0 019	0 016	0 032
A_2	0 045	0 071	0 019	0 039		0 039	0.026	0 019	0 013	.	0 019	0 019	0 013	0 019
B_1	0 019	0 032	0.019	0 026	0 019	0 039	0 019	0 019	0 013	0 019	0 019	0 019	0 013	0 019
B_2	0.019	0.019	0 019	0.019	0 019	0 026	0 019	0 013	0.013	0 013	0 019	0.013	0 013	0 019
C	0 013	0 013	0.013	0 013	0 013	.	...	...	0 013	0 013	.	0 013	0 013	0 013

* Fertilizer treatment:

2A and 2B—Muriate of potash, 160 lbs. to acre.
3A and 3B—Superphosphate, 320 lbs. to acre.
7A and 7B—Nothing.
9A and 9B—Complete fertilizer (N as $NaNO_3$).
10A and 10B—Complete fertilizer (N as $Ca(NO_3)_2$).
11A and 11B—Complete fertilizer (N as $(NH_4)_2SO_4$).
18A and 18B—Complete fertilizer and manure (N as $NaNO_3$).

Table 3 shows the manganese content of profile samples from certain of the soil fertility plots where the fertilizer treatment has been varied over a period of 27 years. A study of the table does not indicate any definite relationship between the percentage of manganese in the soil and the fertilizer treatment. For example, plot 9A, which receives a complete fertilizer but no manure, shows slightly more manganese than plot 18A, which receives the complete fertilizer

plus 16 tons of manure annually. Since manure contains some manganese the soil from plot 18A might be expected to contain more manganese than that from plot 9A. It is probable that the amount of manganese in the manure used is too small to effect sufficient change in the soil to be detected by a chemical analysis.

Attention may be directed to the fact that in the majority of cases manganese is lower in the limed than in the unlimed soils. It is possible that this may be

TABLE 4

Crop yield, manganese in the soil and in the crop

(Sassafras loam with variations in lime treatment)

PLOT NUMBER	LIME TREATMENT (ACRE BASIS)	pH OF SOIL*	YIELD OF HAY		MnO IN THE CROP		MnO IN SOIL
			Alfalfa—1933				
			Second cutting	Third cutting	Second cutting	Third cutting	
			lbs. per acre	*lbs. per acre*	*per cent*	*per cent*	*per cent*
28	No lime	4.6	52	575	0.018	0.096	0.052
29	1000 lbs. Ca limestone	5.1	434	1138	0.020	0.055	0.052
30	2000 lbs. Ca limestone	5.3	1525	2120	0.018	0.025	0.036
31	4000 lbs. Ca limestone	5.8	2176	2150	0.009	0.022	0.046
			Mixed grasses—1935 and 1936				
			1935	1936	1935	1936	
35	No lime	4.9	2100	984	0.064	0.090	0.044
36	1000 lbs. Ca limestone	5.2	2588	1253	0.098	0.072	0.041
37	2000 lbs. Ca limestone	5.5	3290	1909	0.046	0.042	0.041
38	4000 lbs. Ca limestone	6.4	3924	2660	0.033	0.027	0.036
			Wheat—1935				
			Grain	Straw	Grain	Straw	
E	No lime	4.8	2347	3345	0.007	0.017	0.058
F	1000 lbs. Ca limestone	5.1	2828	4455	0.006	0.016	0.071
G	2000 lbs. Ca limestone	5.3	2876	4396	0.004	0.004	0.058
H	4000 lbs. Ca limestone	6.4	3228	4828	0.003	0.003	0.058

* Determined for alfalfa and wheat plots in 1934 and for mixed grass plots in 1936.

due to the fact that in most cases larger crops have been taken from the limed than from the unlimed plots and therefore more manganese has been removed. In certain other cases, however, liming has not made a significant difference in the manganese content of the soil.

With slight exception the A_p horizon contains more manganese than the A_2 or the B_1 horizon. From B_1 to C the difference is generally slight. The higher percentage of manganese in the A_p horizon may be due in part to the higher content of organic matter in this horizon.

Table 4 reports results obtained on samples from experimental plots which have been, for a long time, under different treatments with respect to lime. In this case the lime treatment, the pH, the crop yield, and percentage of manganese in the crop are given, as well as the percentage of manganese in the soil. The figures do not show a definite relationship between the lime treatment of the soil and the percentage of manganese in the soil. There is, however, a very interesting relationship between the lime treatment and the percentage of manganese in the *crop*. With slight exception, the percentage of manganese in the crop is highest with no lime, and decreases with the increase in the amount of lime applied. The explanation is no doubt to be found in the fact that heavy liming would tend to convert the soluble manganese into a less available form, and it is quite probable that under such conditions plants would absorb less manganese than they would if grown in a more acid soil. The fact that the crop yield has increased with increase in the amount of lime applied may possibly be taken as an indication that the yield has not been affected by the lowered intake of manganese. Further work will be done along this line.

SUMMARY

Manganese has been determined in soils representing the more important types found in New Jersey.

In general, the percentage of manganese is higher in soils from the northern part of the state than in those from the southern part, that is, those south of the line connecting New Brunswick and Trenton. The manner in which the soils of the two sections of the state have been formed undoubtedly has something to do with the difference in manganese content.

Long-time manure, fertilizer, and lime treatments do not appear to have influenced the manganese content of the soil. In soils from certain of the experimental plots a rather distinct difference was found between the manganese content of the surface soil, that is the A_p horizon, and the A_2 and B horizons, the amount generally decreasing from the A_p to the B_1 horizon.

Manganese was also determined in certain crop samples from plots that have received no lime and also from plots that have been moderately and heavily limed. With slight exception, the manganese content of the crop decreased with increase in the amount of lime applied. In explanation of this, it is suggested that with heavy liming the manganese is rendered less available, and therefore the crop takes up less of this element. The lowered intake of manganese by the plant does not appear to have a deleterious effect with reference to yield.

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THE INFLUENCE OF CERTAIN REPLACEABLE BASES IN THE SOIL UPON THE ELEMENTAL COMPOSITION OF VEGETABLE CROPS

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A review of the literature reveals that the elemental composition of plant material influences the well-being of animal life. A further study shows that several factors—soil type (1, 5, 6, 13, 14), plant species (11, 13, 15), climatic conditions (2, 3, 4, 9), age of plant (11), and others (1; 10; 12, p. 232–307; 16)—influence the composition of plant material. This paper deals with the effect of certain replaceable bases in the soil upon the elemental composition of some vegetable crops grown under controlled conditions.

EXPERIMENTAL PROCEDURE

Three distinctly different virgin soil types, a Portsmouth loamy fine sand (13.50 per cent organic matter and 274 m.e. exchange capacity per thousand grams of soil), a Bladen sandy loam (1.76 per cent organic matter and 102 m.e. exchange capacity), and a Norfolk fine sand (1.00 per cent organic matter and 48 m.e. exchange capacity) were brought to the greenhouse, screened, and placed in 2-gallon "coffee urn lining" pots. The soils were treated with various bases, and several crops were grown in them before the crops here mentioned were planted. These data have been given in various other publications (7, 8). This phase of the subject will not be discussed, since this paper deals only with some of the soil factors that influence the elemental composition of the plant material. It is sufficient to mention that in addition to the treatment shown in the tables, the pots were all fertilized with a basic application of nitrogen, phosphorus, and potash. These materials were given in several applications as the plants increased in size. Five plants in each case were grown in each pot, and the figures given are for two or more closely agreeing pots. The analyses for the nutrients leached were obtained from 1500 cc., or the equivalent of about 2 inches of rain water leached through the soil at the end of the growing season. The soils were then sampled and the replaceable bases determined. These results together with the analyses of the plants are given in table 1.

COMPOSITION OF PLANT MATERIAL

Plants exhibit certain selective powers for absorbing nutrients (12, p. 232–307) but are greatly influenced by the composition of the soil solution. While

TABLE 1

The influence of soil type and calcium lime upon the composition of the lima bean plant

pH OF SOIL	PER CENT BASE SATURATION pH 7.0	FREE CaCO₃	REPLACEABLE*			Leached†				DRY WEIGHT, GRAMS		M.E. PER GRAM DRY MATTER									
		M.e. per 1000 gm. soil	M.e. per 1000 gm. soil			M.e. per 1000 gm. soil						Vegetation					Pods				
			Ca	Mg	K	Ca	Mg	K	N	Tops	Pods	Ca	Mg	K	N	P	Ca	Mg	K	N	P
Portsmouth Soil																					
4.0	10	....	27	11 2	15 4	2.00	0 62	1.37	6 48	9 1	5 3	0 36	0 30	1.41	2.82	0.43	0.10	0.17	0.70	2.56	0.23
5.1	41	....	114	13.4	7 4	0.32	0 73	0.08	0 37	26 5	43.5	1.56	0.32	0.56	1.10	0.38	0.10	0.15	0.48	1.58	0.16
6.1	74	6	202	9 9	6.5	0.55	0.34	0 06	0 09	36 4	52 5	2 00	0.20	0.34	1.04	0.29	0.16	0.20	0.63	1.60	0.28
6.8	97	18	267	11.7	8.8	0.53	0.12	0 08	0 05	36 6	47.1	2 06	0.20	0.41	1.04	0.28	0.18	0.24	0.61	1.76	0.30
7.0	107	28	294	11.7	8.3	0.96	0.13	0.29	0 09	36 8	45.7	2 48	0.19	0.48	1.24	0.26	0.20	0.24	0.63	1.86	0.30
Bladen Soil																					
4.0	15	....	16	10 8	16.2	3 22	0.75	1.93	5 22	. .	....	. .	. .	..	....	....	.....	.....	.....	.....	.....
5.6	47	....	48	15 9	6 1	0 65	0.15	0.05	0 07	29 2	49 7	2 04	0.45	0 41	1.14	0.30	0.02	0.38	0.61	1.70	0.34
6.6	80	8	81	14 2	6 0	0.78	0 14	0 05	0 02	33 7	51 3	2 26	0.34	0 34	1.10	0.27	0.08	0.36	0.59	1.74	0.34
7.2	123	44	126	15.9	4 9	0.53	0.20	0 04	0 04	41 8	49.7	2 22	0.34	0 20	1.20	0 27	0.20	0.29	0.59	1.84	0.32
Norfolk Soil																					
5.0	28	....	13	9 8	3.9	1 72	0.40	0 96	0 92	27 9	32.0	0.46	0 28	1 07	1.96	0.36	0.08	0.24	0.71	2.24	0.30
5.5	37	....	18	15.0	3.8	1 08	0 37	0.29	0 14	29 5	38 8	0 88	0 55	0 65	1.30	0 44	0 04	0.35	0.68	1.78	0.35
5.7	48	....	23	14 7	4 1	0.84	0 38	0 15	0 08	30 7	44.7	1 02	0 75	0.51	1.06	0 38	0.18	0.30	0.61	1.66	0.32
6.1	81	....	39	11.0	4 3	1.54	0 32	0 10	0 08	35.3	42 4	1 36	0.48	0.43	0.92	0 38	0.18	0.27	0.65	1.86	0.32
7.0	118	40	57	9 2	4 5	2 14	0 17	0.14	0 08	48 1	41.6	1 54	0.31	0.43	0.72	0.31	0.22	0.26	0.66	1.54	0.30

* Determined by leaching 25 gm. of soil with 500 cc. of 0.5 *N* ammonium chloride (after crop grown).

† Determined on leaching at the end of cropping period (1500 cc. per pot).

TABLE 2

The influence of different bases upon the composition of the lima bean plant in Portsmouth soil

	pH of soil	Per cent base saturation pH 7.0	Free $CaCO_3$	Replaceable*			Leached†				Dry weight, grams		M.E. per gram dry matter									
			M.e. per 1000 gm. soil	M.e. per 1000 gm. soil			M.e. per 1000 gm. soil						Vegetation					Pods				
				Ca	Mg	K	Ca	Mg	K	N	Tops	Pods	Ca	Mg	K	N	P	Ca	Mg	K	N	P
	4.0	10	. ..	27	11 2	15 4	2 00	0 62	1.37	6 48	9.1	5 3	0.36	0.30	1 41	2 82	0 43	0.10	0 17	0.70	2.56	0.23
High calcium	6 1	74	6	202	9 9	6 5	0 55	0 34	0.06	0 09	36 4	52 5	2.00	0.20	0 34	1 04	0 29	0.16	0.20	0.63	1.60	0.28
High magnesium	6.1		4	68	70 6	7 3	0.30	0 17	0.04	0 23	35 6	45 4	0 94	1 36	0.44	1 22	0.29	0 14	0 42	0 66	1.82	0.36
Calcium and magnesium	6.0		....	124	56 2	6 2	0 20	0 15	0.08	0 07	35 6	43 9	1 62	0 61	0.44	1.28	0 27	0.18	0 37	0.63	1.98	0.33
High potassium	5.6		....	93	12 6	33.0	0.57	0.11	1 44	0 09	41 9	45.6	0.92	0 17	1 36	1 36	0.25	0.10	0 14	0.87	2.04	0.19
Calcium, magnesium, and potassium	5.9		...	116	54.5	15.2	0 39	0 16	0 21	0.02	40.6	42 9	1 02	0 63	0 54	1.02	0.32	0.12	0.34	0.70	1.56	0.30

* Determined by leaching 25 gm. of soil with 500 cc. of 0.5 *N* ammonium chloride (after crop grown).

† Determined by leaching at the end of cropping period (1500 cc. per pot).

the replaceable calcium in the Portsmouth soil varied from 27 to 294 m.e. per thousand grams of soil, the vegetative material grown on this soil varied from 0.36 to 2.48 m.e. The calcium leached was greater for the no-calcium treated pots than for the high-calcium treatment. This indicates that there was not an actual calcium shortage in the no-calcium treated pots but that other interfering factors limited its absorption. Unabsorbed (plant) anions (nitrates, chlorides, sulfates, etc.) largely account for the leaching of replaceable bases. Although the replaceable potassium and magnesium were about the same in each of the pots (table 1) at the beginning of the experiment, the greater absorption by the larger plant growth on the limed soils somewhat changed that found

TABLE 3

The influence of different bases upon the composition of collards

	pH OF SOIL	LEACHED M.E. PER 1000 GM. SOIL				DRY WEIGHT, GRAMS	M.E. PER GRAM DRY MATTER				
		Ca	Mg	K	N		Ca	Mg	K	N	P
Portsmouth Soil											
High calcium	4.0	2 91	0.93	1.47	5 95	3.1	0 38	0 22	1 13	3 30	0.53
	6.0	0 51	0.21	0 04	0 14	35 6	1 48	0 19	0 97	1 68	0 45
High magnesium	6 4	0 51	0 35	0 02	0 74	35 4	1 00	0 66	1 22	2 06	0.61
High potassium	5 6	0 56	0.51	0 41	0 08	34 4	1.10	0.27	2 27	2 38	0 50
Calcium and magnesium	6 5	0.37	0.17	0.02	0 06	40 2	1 16	0.62	0 90	1.88	0 45
Calcium, magnesium, and potassium	6 6	0 54	0.22	0 09	0.14	38.5	0.74	0 43	1.10	1 42	0 43
Bladen Soil											
	4 3	4.07	1.11	1.37	4 78	1.8	0 86	0.32	1 71	4 34	0.38
	5 6	0 76	0.12	0.06	0 14	37.4	1.00	0.27	1.06	1.12	0 49
Norfolk Soil											
	5 3	1.75	0 42	2.10	4.54	1.6	0 69	0.27	1.59	3 66	0.58
	5.9	0.55	0.16	0.02	0.00	32.5	0.92	0.43	1 04	1.36	0.52

after the harvest of the crops. The magnesium content of the plant material varied somewhat on the different calcium treated soils but not as greatly as did the potash and nitrogen. The pods showed a lesser variation for all of the elements, as has been previously reported in the literature.

The analyses of the plant material from the Bladen and Norfolk soils are similar to those of the Portsmouth soil but are less pronounced. This is perhaps due to the lesser variation in the amounts of replaceable lime required to bring the soils to a neutral reaction. Consequently, there was a lower ratio of calcium to the other bases in these two soils. One noticeable difference was in the low calcium content of the pods at the low pH values.

INFLUENCE OF CERTAIN BASES UPON ABSORPTION OF OTHER PLANT NUTRIENTS

In the original soil treatment of the Portsmouth series, a part of the calcium carbonate was replaced with magnesium and potassium carbonate in changing the soil reaction to a condition more favorable for plant growth; and the influence of the added element upon the composition of the plant was determined. These data, given in table 2, support the data in table 1 in showing that a particular base in the soil greatly influences the absorption of the plant nutrients. For example, a high replaceable calcium content in the soil suppresses the absorption of potassium, nitrogen, and magnesium by the plant; a high replaceable magnesium content suppresses the absorption of potassium, calcium, and nitrogen; and a high potassium content suppresses the absorption of calcium, magnesium, and nitrogen.

COMPOSITION OF COLLARDS

Collards were grown on the variously treated soils and were harvested at maturity or at about the time they would have been cut for consumption. The chemical analyses of certain plant materials are given in table 3. From these analyses it is learned that even though the yields were not greatly influenced by a certain treatment, such as high magnesium or high calcium, the elemental content of the added mineral was greatly increased at the expense of the absorption of other elements.

SUMMARY

The presence of a large amount of a particular replaceable base in the soil colloidal complex influenced the elemental composition of plant material even though the yields were affected but little. A high replaceable calcium content suppressed the absorption of potassium, nitrogen, and magnesium; a high replaceable magnesium content suppressed the absorption of potassium, calcium, and nitrogen; and a high potassium content suppressed the absorption of calcium, magnesium, and nitrogen.

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OXYGEN AND CARBON DIOXIDE CONTENT OF CULTURE SOLUTIONS IN RELATION TO CATION AND ANION NITROGEN ABSORPTION BY TOMATO PLANTS[1]

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The necessity of adequate soil aeration for the normal healthy root development of most species of mesophytic plants has for some time been considered as due largely to the favorable supply of oxygen which is thus furnished by the soil atmosphere. Since the percentage volume of soil air to be found at any given time or place is variable, being dependent chiefly upon the physical nature of the soil, the size of its particles, and especially its water content, the supply of oxygen may thus become a limiting factor in the growth and development of roots under certain conditions.

Furthermore, as Cannon (5, 6) has shown, different species growing under the same conditions may vary appreciably as to their oxygen requirements for root growth; and the requirements of any given species may in turn vary according to the temperature of the root medium.

In the case of solution cultures, the problem of maintaining a favorable oxygen supply in the culture medium becomes of even greater importance than under soil conditions, since the roots of large, rapidly growing plants are apt to extract oxygen from the solution more rapidly than it can be absorbed by the solution from the atmosphere. To offset this condition of oxygen deficiency, artificial aeration of culture solutions has been employed in various instances, usually with marked success.

Sachs (16), in his pioneer work with nutrient solutions as early as 1860, encountered this problem with some of his cultures, notably those whose roots completely filled the culture jars, and these he aerated with beneficial results. Since then, others in increasing numbers have repeatedly demonstrated the desirability of aerating culture solutions when employed in experimental studies with plants.

Arker (4), in 1901, reported that growth of lupine roots was more rapid, in both soil and water cultures, when a stream of air was passed through the culture medium than it was without artificial aeration. Using the same species and also barley, Hall, Brenchley, and Underwood (11) later obtained a 50 per cent increase in total dry weight of plants grown with aeration over those grown without.

Allison and Shive (1) made a thorough study of the effect of aeration on the

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growth of soybean plants in sand and water cultures, with and without continuous renewal of solution. They obtained greater growth of both roots and tops with aeration than without, in the cultures with continuous solution renewal; but with only periodic renewal of solution, the aeration benefited the root growth of the plants, but not appreciably the top growth. More recently Clark and Shive (7) have obtained beneficial results of aeration with tomato plants, using the constant drip method of continuous solution renewal.

In a very significant and important study recently reported, Loehwing (14) states that continuous soil aeration with moist air increased size and growth rates of plants, but very rapid aeration had the opposite effect. This indicates the possibility of setting up, under certain conditions, excessive soil oxygen tensions which might tend to inhibit rather than to promote plant growth.

While the influence of oxygen has generally been regarded as of paramount importance in the relationship of aeration of culture media to root growth, the possibility has been suggested by some authors that the carbon dioxide content of the solution may also be involved in this relationship. When the high solubility of this gas in an aqueous medium and the relatively high rate of respiration of the roots of vigorously growing plants are considered, it is not surprising that the quantity of carbon dioxide present in culture solutions is frequently found to be far in excess of that normally present in the atmosphere.

There is evidence to show that relatively high concentrations of carbon dioxide, such as may be found in culture solutions, may be decidedly injurious to plant growth. It has been shown by Free (10) that bubbling carbon dioxide through culture solutions containing buckwheat plants produced injury in several hours and caused the death of the plants in a few days, whereas the same treatment with air, oxygen, or nitrogen produced no injurious effects. Knight (13), using the same treatment with corn plants in solution cultures, found a better correlation of the rate of root growth of the plants with the carbon dioxide content of the solutions, in an inverse ratio, than with the oxygen content in a positive ratio. From this he concluded that carbon dioxide and not oxygen was the limiting factor in the growth of the roots in his experiments.

It is quite possible that the aerating treatment such as was used in the present experiment might influence the carbon dioxide content of the solution by sweeping out some of the dissolved gas along with the air bubbles constantly passing through the solution. Accordingly, in connection with this study dealing with the relation between cation and anion nitrogen absorption by the tomato plant and the oxygen level of the culture solutions, a study was also made of the effect of the carbon dioxide content of the solution on the rates of nitrogen absorption under consideration and on growth yields, in order to determine whether or not this factor might play a significant part in the relation between aeration and ion absorption.

EXPERIMENTAL METHODS

Tomato plants of the variety Marglobe were grown in solution cultures for all the experimental work here described. The plants were obtained from seed

of a selected strain, which produced individuals having a reasonable degree of uniformity in both type of plant and vigor of growth.

Solution

The culture solution used throughout the growth period of the plants, as well as during the experimental test intervals, was one of the Tottingham type, a modified four-salt solution of the ammonium sulfate series used and described by Jones and Shive (12). This solution had previously been found to give good growth of the tomato in water cultures; moreover, since it contained approximately equivalent concentrations of nitrogen as ammonium and as nitrate, it was well suited for use in testing the comparative rates of absorption of these two forms of nitrogen by the plants.

The solution was first used at a concentration of 0.1 atmosphere for the very young plants, and the concentration was increased successively to 0.5 and then to 1.0 atmosphere, as the plants increased in size. The pH of the solution was adjusted by the use of normal potassium hydroxide and sulfuric acid, the pur-

TABLE 1

Milliliters of half-molar stock solutions, used in making up 1 liter of culture solution at three concentrations

STOCK SOLUTIONS	0.1 ATM.	0.5 ATM.	1.0 ATM.
KH_2PO_4	0 42	2.12	4 23
$MgSO_4$	1 42	7 12	14 23
$(NH_4)_2SO_4$	0 56	2 80	5 60
$Ca(NO_3)_2$	0 58	2 92	5.84

pose of which will be stated. The various constituents and the quantities of each of the salts which were used in making up the solution, at the different concentrations are given in table 1.

Boron and manganese, in quantities of 0.5 p.p.m. each, were supplied to the plants in all series, in the form of boric acid and of manganese sulfate, respectively. Iron, in the form of ferrous sulfate, in solution made up fresh at every application was supplied at the rate of 0.5 to 3 p.p.m. of iron, according to the needs of the plants.

Growing the plants

Seeds were germinated in shallow boxes of clean washed white sand and grown there for from one to two weeks after germination. During this period the young plants received frequent applications of the culture solution, sprinkled on the surface of the sand, at a concentration 0.1 atmosphere and at pH 5.3.

When the seedlings were about 4 cm. high, the most uniform individuals were selected and transferred from the sand to culture solutions in 2-quart fruit jars of colorless glass, three plants to a jar. These were supported at the

top of the jar, after the method used and described by Tottingham (20). The plants in all cases remained in these supports to the completion of the experiments.

After the plants had grown to considerable size, they were transferred together with their supports from the 2-quart containers to 4-quart containers. This allowed for a greater volume of solution in continual contact with the roots and also provided more room for the rapidly growing root systems.

When the young plants were first transferred from sand to the culture jars the solutions were renewed at sufficiently frequent intervals to prevent any marked change in pH until the plants had attained a height of about 15 cm. During this preliminary growth period, the solution of each culture was kept as nearly as possible at pH 5.3, but the total concentration of the solution was gradually increased from 0.5 atmosphere to 1.0 atmosphere.

At this time the technique for the main part of the experimental period was instituted and it was maintained to the end of the experiments, when the plants were tested and then harvested. This technique included (*a*) the continuous flow according to the method described by Shive and Stahl (18), (*b*) use of the solution at two different pH levels (one group of plants at pH 4 and the other group at pH 7), and (*c*) artificial aeration of each individual culture. At first each culture received the solution at the rate of 1 liter in 24 hours, and later at double the rate.

The purpose of growing and testing these plants at the two different pH levels may be briefly explained by referring to the work of Clark and Shive (8), who showed that the pH of the culture solution exerts a significant influence on the rates of absorption of ammonium and nitrate ions by the roots of plants. Thus, in the present work it was deemed essential to take into consideration this important factor.

The stimulating effect of artificial aeration of the culture solution on the growth made by vigorous plants of the type and species here used has also been well brought out by Clark and Shive (7). A more specific study of the nature of this influence on one factor concerned in the growth of plants, namely, the rates of absorption by the plants of the two forms of nitrogen from the culture solution, is the chief purpose, of course, of the present work, in the experiments about to be described.

Aeration of these cultures was effectively accomplished by the method previously used by Allison and Shive (1), but their apparatus was here modified for use on a much more extensive scale than was required for their purpose.

Air was admitted through a glass tube at the bottom of the culture jar as a jet of small bubbles passing up freely through the solution. The rate of bubble flow was regulated to deliver approximately 10 bubbles per second. The size of bubbles in the various cultures was fairly uniform, since the glass tubes were selected for uniformity of bore.

During the course of the experiments a comparison was made also between cultures which received continuous artificial aeration and those which received

none at all, with respect to the growth made and the general condition of the plants under the two treatments.

Testing the plants

When the plants had attained the desired size and the roots had completely filled the culture jars in which they were growing, tests were conducted. Each culture of the series was tested twice, with an interval of two days between the tests. The first of these tests aimed to determine the amount of oxygen and of carbon dioxide in solution, after an interval of 6 hours in contact with the plant roots under the several conditions of aeration and at the two levels of pH employed. The second test was then conducted with the same plants for the purpose of determining the rates of absorption of the nitrate and ammonium ions by the roots, under the same conditions of aeration and pH. All tests were carried out, using the same technique, during the 6-hour interval in the middle of the day and in bright sunlight. The cultures were arranged in the greenhouse to receive as nearly as possible uniform light and atmospheric conditions throughout the test period.

In each test, carried out in duplicate, four different groups of plants were involved as follows: (*a*) aerated at pH 4; (*b*) aerated at pH 7; (*c*) non-aerated at pH 4; and (*d*) non-aerated at pH 7.

Each culture of the four groups of plants was removed from the solution in which it had been growing, thoroughly washed by successive rinsings in five separate vessels of distilled water, and replaced in 3750 ml. of the test solution, accurately measured, in clean culture jars. This solution was identical in every respect with that in which the plants had previously been growing. Moreover, 500 ml. of fresh solution was flowed through each culture during the 6-hour test period by the method previously used and at the same rate as that employed prior to the test. Thus, 4250 ml. of test solution was used altogether.

Each culture was subjected to its respective treatment of aeration or non-aeration, as before described, as soon as it was placed in the test solution. In each test the cultures were started at intervals of from 10 to 12 minutes, in order to allow sufficient time for uniform treatment at the end of the test. This treatment consisted in collecting, for oxygen and carbon dioxide analyses, samples of the solutions from each of the culture jars at the end of the 6-hour test interval.

All test solutions used throughout the experiments were made up and exposed to the atmosphere for several days prior to their use, in order that they might come into equilibrium with the atmosphere, thus insuring as far as possible at the beginning of the test the same quantity of oxygen and of carbon dioxide in the solutions of all of the cultures. Duplicate samples of the solutions were taken just prior to each test, in order to determine the quantity of oxygen and of ammonium and nitrate nitrogen in the solution before it was exposed to the roots of the plants.

At the end of this first test period, each culture, after being sampled, was

restored to its former status for a period of two days, when the second test was begun.

The treatment of the cultures after the test period for absorption consisted in thoroughly washing the test solution from the roots of the plants in each culture with a fine stream of distilled water directed for a wash bottle, and in rinsing all glassware which had been in contact with the solution. Then, all solution and rinsings from each individual culture were collected in vessels large enough to allow for making up the solution to desired volume. Before being made up to volume, however, the solutions were first filtered through Whatman No. 4 filter paper, to remove any solid particles of organic matter which might alter the nitrogenous content of the solutions in the interim before the analyses could be made.

The plants of each culture were harvested directly after the absorption test was completed, and the green and dry weights of both stems and leaves were separately determined.

ANALYSES OF THE TEST SOLUTIONS

Oxygen

To determine the oxygen content of the samples of solution which were collected at the end of the 6-hour test periods, use was made of the Micro-Winkler method for determining dissolved oxygen, as proposed by Lund (15) with modifications as required. Further details of this procedure may be found in any standard work on volumetric analyses, such as that by Sutton (19).

Samples were collected directly from the culture jars in 50-ml. sample bottles, prepared according to the method used and described by Allison and Shive (2), by means of which the solution was transferred from the culture jar to the sample bottle without exposure to the atmosphere. With a few exceptions, all analyses herein recorded were run within a few minutes after sampling. In no case were the samples allowed to stand for any considerable period of time before analysis, and at all times they were carefully sealed from all contact with the atmosphere. Duplicate determinations were always made, and the oxygen content was calculated directly in terms of parts per million of oxygen.

Carbon dioxide

Samples of the test solution for carbon dioxide determinations were collected in 100-ml. sample bottles of the same type and in the same manner as for the oxygen analyses. The same necessity here of preventing exposure of the solution to the atmosphere as in the case of the oxygen samples is apparent. The method and the apparatus used in conducting the carbon dioxide analyses have been described in detail in another publication (4).

Ammonium and nitrate nitrogen

The method used for nitrogen analyses was a modification of the Folin aspiration method, worked out and described by Sessions and Shive (17). In-

stead of the sodium carbonate and sodium chloride used by them for the liberation of ammonia from the solutions, 5 ml. of 13 per cent sodium hydroxide per 100-ml. sample, as described by Clark (9), was here used, to bring the concentration of the sample slightly higher than 0.125 *N* NaOH. For nitrate recovery, Devarda's alloy, 2.5 gm. per 100-ml. sample, as used by Sessions and Shive, was added without the further addition of alkali.

EXPERIMENTAL RESULTS

The data here presented embody the results obtained with a series of plants, the cultures of which were started in September, grown during a period of 60 days and tested and harvested in November.

In this presentation direct comparison will always be made between the cultures which were aerated and those which were not aerated. Results of these two treatments will be compared separately for each of the two pH levels at which the plants were tested. Moreover, the consideration of the rates of absorption of nitrogen under the conditions of these treatments will always be taken up from two separate standpoints, (*a*) the absorption of ammonium (NH_4^+) ions and (*b*) the absorption of nitrate (NO_3^-) ions, for each of the four sets of conditions as just stated.

Oxygen content of solutions as influenced by contact with plant roots

The quantities of oxygen contained in these solutions before and after they had been in contact with the roots of the plants for the 6-hour test period, are presented in table 2. Since two separate stock supplies of solution were always prepared for these tests, one made up to pH 4.0 and the other to pH 7.0, the oxygen content before the tests was determined separately for each supply, after the solutions had been standing exposed to the atmosphere for a number of hours. The two values, averages of duplicate determinations, are here included. The oxygen content of all lots of solution thus determined before the tests showed agreement which was well within the limits of experimental error.

The most significant feature brought out in table 2 is the difference between the oxygen content of the solutions before and after the 6-hour test periods during which the plant roots were immersed in the solutions. Even under the influence of the continuous and vigorous aeration as here carried out, the oxygen content of the solutions expressed as the average for the cultures of like treatments at the end of the test period was only 14.9 per cent of the original content. The corresponding percentage value for the non-aerated cultures of the two series was very much less than that for the aerated, as might be expected, being only 3.6 per cent of the original oxygen content of the solutions.

This extreme depletion of the solutions of their available oxygen supply by vigorously growing plants in such a relatively short period of time is characteristic of all the non-aerated cultures tested in these experiments. The fact that the aerated cultures contained, in absolute amounts, less than 1 p.p.m. more of oxygen than the non-aerated, leads to the conclusion that even under the influence of the rapid aerating process here employed, the actual rate of

removal by the plants exceeds the solubility rate and is definite evidence of the pronounced deficiency in the available supply of this element in the non-aerated cultures. That such deficiency is directly correlated with retarded growth rates becomes clear from a consideration of yield values. In view of this fact it is reasonable to assume that any external factors which would significantly affect the supply of oxygen in the growth media would in turn have their corresponding effects on the vital processes of the roots. Of these factors, that of ionic absorption must necessarily be one of primary importance.

Without exception, the aerated cultures showed appreciably higher oxygen content than the corresponding non-aerated cultures.

TABLE 2

Oxygen content of solutions, before and after contact with plant roots during an experimental interval of 6 hours, at two pH levels

	ORIGINAL O_2 CONTENT	O_2 CONTENT AFTER CONTACT WITH PLANTS DURING 6-HOUR INTERVALS		RATIO $\frac{\text{AERATED}}{\text{NON-AERATED}}$
		Aerated	Non-aerated	
	*p.p.m.**	*p.p.m.**	*p.p.m.**	
pH 4.0	8.03	1 56	0.35	
		1.02	0.26	
	7 98	1.23	0.31	
		0 96	0 22	
Average	8 01	1.192	0 285	4.18
pH 7.0	7.95	2 20	0.37	
		1.87	0 46	
	8.11	0 62	0 56	
		0 55	0 39	
Average	8.03	1 310	0 445	2.94

* Or mgm. O_2 per l. of solution.

The exact numerical relationship between the average results of the two treatments, with and without aeration, may be represented as the ratio of the average value for the aerated to the corresponding value for the non-aerated cultures, as given in table 2. These ratio values show that the average oxygen content of the aerated cultures at pH 4.0 was 4.18 times that of the non-aerated cultures at the end of the 6-hour test period, whereas at pH 7.0 the average oxygen content of the aerated cultures was 2.94 times that on the non-aerated cultures.

The fact that the actual quantity of oxygen contained in any of the solutions at the end of a 6-hour interval, even under the influence of the aerating treatment, was relatively small with respect to the saturation point of the gas in solution under these conditions, does not alter the significance of the relationship just mentioned. Obviously these values, representing parts per million

of oxygen contained in solutions at the end of the 6-hour test period, give no indication of the total quantity of oxygen absorbed by the plants of any culture over the course of the 6-hour period. This of necessity follows from the nature of the experimental set-up, which does not permit a measurement or even an approximation of the actual quantity of oxygen dissolved by the solutions of either the aerated or the non-aerated cultures during the 6-hour test period, and hence the total oxygen taken from the solutions of the plant can not be estimated. It may reasonably be assumed, however, that the plants in the aerated cultures do absorb oxygen from solution at a higher rate than do the plants in the non-aerated cultures, since the roots of the former are continuously exposed to a higher oxygen potential than are those of the latter and since an enormously greater total volume of air is exposed to the solvent action of the aerated than to that of the non-aerated solution during this period.

Nitrogen absorption in relation to aeration

The numerical data relating to nitrogen absorption rates as influenced by aeration of the culture solutions are presented in table 3.

Comparison of the nitrogen absorption values for the aerated and non-aerated cultures shows a consistently higher rate of absorption of both ammonium and nitrate nitrogen per unit of plant tissue per unit of time from the aerated than from the corresponding non-aerated solutions. The importance of aeration as a factor influencing the nitrogen absorption rates here indicated is emphasized by the consistency with which this relationship holds at the two pH levels employed. Although there was an appreciable difference in the size of the plants at the two different pH levels, the plants of the aerated cultures at each pH level showed a higher absorption rate than did the plants of the corresponding non-aerated cultures.

The ratios of the average absorption rate values indicated for the aerated cultures to those indicated for the non-aerated cultures are given in the last two vertical columns of table 3. These ratios show that the average rate of absorption of NH_4—N from the aerated cultures at pH 4.0 was 2.29 times as high as the corresponding rate from the non-aerated cultures, and at pH 7.0 the rate was 1.34 times as high from the aerated cultures as it was from the non-aerated. The average NO_3—N absorption rate at pH 4.0 and at pH 7.0 is shown to be 1.31 and 1.30 times as high, respectively, from the aerated cultures as it is from the non-aerated.

Nitrogen absorption in relation to pH of the culture solutions

Consideration of the nitrogen absorption rates in relation to pH values very clearly brings out the fact that this solution factor itself exerted a very considerable influence on the actual rates of absorption of both the ammonium and the nitrate ions from the solution. In no instance, however, does this affect the general nature of the relationship between the aerated and the non-aerated treatments. The average rates of absorption were always higher in

the aerated than in the corresponding non-aerated cultures, regardless of the incidental effect of the pH of the culture solutions on corresponding absorption rates.

Thus, the ratios given in the last two horizontal lines of table 3 show that the actual average rate of absorption of NH_4—nitrogen from aerated solutions at pH 7.0 was 2.26 times as high as was the corresponding rate from the aerated solutions at pH 4.0, and from the non-aerated cultures at pH 7.0 the average rate of absorption of this ion was 3.85 times as high as it was from the corresponding cultures at pH 4.0. On the other hand, the average absorption rate of the NO_3—nitrogen from the aerated solutions at pH 4.0 was 1.69 times as high as it was from the aerated cultures at pH 7.0, and from the non-aerated cultures at pH 4.0 the average rate was 1.68 times as high as it was from the corresponding cultures at pH 7.0. These results are in entire accord with the previous work of Clark and Shive (9).

TABLE 3

Ammonium and nitrate nitrogen absorbed during a 6-hour experimental interval from aerated and non-aerated culture solutions at two pH levels

	MGM. OF N PER 100 GM. GREEN TISSUE				RATIO OF ABSORPTION FROM CULTURES $\frac{\text{AERATED}}{\text{NON-AERATED}}$	
	NH_4—N		NO_3—N		NH_4—N	NO_3—N
	Aerated	Non-aerated	Aerated	Non-aerated		
pH 4.0	4 01	2.45	8.93	8.11		
	3 91	1 89	8 22	7 41		
	3 40	1 10	11 39	6 65		
	3.72	1 13	9 97	7 14		
Average	3 760	1.642	9.628	7 327	2 29	1 31
pH 7.0	10 21	7 64	9 79	5 21		
	9 09	5 99	5 42	5 72		
	8 60	5 51	5 53	3.55		
	6 04	6 18	2 03	3 01		
Average	8 485	6 330	5 692	4 372	1 34	1 30
Ratio of average absorption rates: $\frac{\text{pH 4.0}}{\text{pH 7.0}}$	0 443	0.259	1.69	1 68		
$\frac{\text{pH 7.0}}{\text{pH 4.0}}$	2.26	3.85	0.591	0.596		

Weight yields produced by aerated and non-aerated cultures

A comparison was made of the total plant growth produced by the cultures which were continuously aerated with that produced by the corresponding

non-aerated cultures, during the growth period of 60 days. The numerical data relating to the green and dry weight yields of the plants are presented in table 4.

Without exception it was found that the continuously aerated cultures notably surpassed the non-aerated not only in the total amount of plant material produced but also in the character of the growth and general appearance of the plants at each of the two pH levels employed. The marked influence of aeration is clearly shown by the ratios between the average yields produced by the aerated and the non-aerated cultures as given in table 4. These ratios

TABLE 4

Green and dry weights of 60-day-old tomato plants grown with and without artificial aeration and at two pH levels

	GREEN WEIGHT PER CULTURE		RATIO $\frac{\text{AERATED}}{\text{NON-AERATED}}$	DRY WEIGHT PER CULTURE		RATIO $\frac{\text{AERATED}}{\text{NON-AERATED}}$
	Aerated	Non-aerated		Aerated	Non-aerated	
	gm.	gm.		gm.	gm.	
pH 4.0	397	189		28 1	17 2	
	323	163		24 2	14 3	
	364			30 4		
	353			28 8		
Average	359	176	2 04	27 9	15 8	1 77
pH 7.0	477	195		35 7	18 9	
	538	164		40 7	15 8	
	490			36.4		
	445			33 4		
Average	488	180	2 71	36 6	17 4	2 10
Ratio of yields:						
$\frac{\text{pH 4.0}}{\text{pH 7.0}}$	0 74	0 98		0 77	0 91	
$\frac{\text{pH 7.0}}{\text{pH 4.0}}$	1 36	1 02		1 31	1 10	

show that the average green weight yields produced by the aerated cultures at pH 4.0 and pH 7.0 were 2.04 and 2.71 times the corresponding yields produced by the non-aerated cultures, respectively, whereas the average dry weight yields produced by the aerated cultures at pH 4.0 and pH 7.0 are shown to be 1.77 and 2.10 times as high, respectively, as the corresponding yields produced by the non-aerated cultures.

Table 4 further brings out the fact that, regardless of the influence of aeration upon yields, the plants grown in the solutions at pH 7.0 always produced higher yields than did those grown in the corresponding solutions at pH 4.0. The superiority of average yields produced by the plants grown at pH 7.0 over

those grown at pH 4.0 is shown by the ratio values given in the last horizontal line of the table.

This relation between yields and pH of the growth media is in very close agreement with the corresponding relation between average rates of total nitrogen absorption (NH_4—N plus NO_3—N) and pH of the growth media as shown in table 3. Thus it appears that the total vegetative growth made by these plants is, in a measure at least, determined by the rates at which the plants absorb nitrogen, regardless of the factors which influence these rates.

The difference in appearance between the continuously aerated and the non-aerated plants is shown by the photographs of plate 1, which were taken more than two weeks before the time of harvesting the plants. The differences at the time of harvesting had become considerably more pronounced than the photographs show.

As may be seen, one of the most striking differences appeared in the size and type of root systems which were made by the two groups of plants. The aerated cultures produced a profusion of long, white, well-branched roots which at the time of harvesting completely filled the culture jars. In contrast to this vigorous healthy condition, the roots of the non-aerated cultures failed to penetrate the solution to the lower part of the culture jars, but became localized and matted near the surface of the solution. This emphatically indicates the stimulating influence of the oxygen, which here is absorbed by the solution at the surface of the liquid only at a very slow rate.

The difference between the development of the root systems of the plants of the two groups was reflected also in a somewhat similar superiority of the aerated over the non-aerated plants in size, vigor, and general appearance of stems and leaves, which was approximately commensurate with the superiority in green and dry weight yields just noted. Large vigorous stems and an abundance of broad leaves were characteristic of the growth made by the aerated plants, while the non-aerated plants, in contrast, were characterized by small, hard, woody stems, by longer internodes, and by smaller and fewer leaves. Flowers were produced by both the aerated and the non-aerated plants, however, at practically the same time.

Carbon dioxide content of the culture solutions

The solution samples from which the carbon dioxide data were obtained by analyses were taken at the same time and in the same way as were the samples for the determination of oxygen, at the end of the 6-hour test interval. The analyses were made as soon as possible after sampling. The results of these analyses are given in table 5. All values given represent averages of not fewer than duplicate determinations.

Some attempt was made to maintain temperatures of the solutions within a reasonable range of variation during the 6-hour test periods. This was accomplished, as may be seen from the temperature record given in the table; therefore, no important significance can be attached to temperature variations, during these test intervals, as related to experimental results.

It will be observed that there is considerable accumulation of carbon dioxide in the solutions of all cultures, both aerated and non-aerated, during the 6-hour period of contact with plant roots. This accumulation is particularly pronounced in the pH 7.0 solutions, as is indicated by the ratios between the average carbon dioxide content of solutions at pH 7.0 and pH 4.0, respectively, at the end of the 6-hour experimental period. These ratios show the average carbon dioxide content of the solutions of the aerated and non-aerated cultures at pH 7.0 to be 2.07 and 1.82 times as high, respectively, as that of the solutions of the corresponding cultures at pH 4.0. Since such evidence as is available on the influence of carbon dioxide on growth responses indicates that its presence is harmful rather than stimulating, the obvious conclusion is that the superior yields of the pH 7.0 cultures over those of the pH 4.0 cultures in question were produced in spite of high carbon dioxide content rather than because of it.

TABLE 5

Carbon dioxide content of aerated and non-aerated culture solutions at two pH levels, before and after contact with plant roots during an experimental interval of 6 hours

	TEMPERATURE		ORIGINAL CO_2 CONTENT	CO_2 CONTENT AFTER 6 HOURS CONTACT WITH PLANT ROOTS		RATIO $\frac{\text{AERATED}}{\text{NON-AERATED}}$
	Aerated	Non-aerated		Aerated	Non-aerated	
	°C.	°C.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
pH 4.0	21 3	23 3	13.3	17 6	18 0	
	20 8	22.4		15 8	26 6	
Average	21 1	22.9		16 7	22 3	1 34
pH 7.0	21 5	21 6	13.9	34 8	42 1	
	20 5	22 0		34 2	38 8	
Average	21 0	21.8		34 5	40 5	1 21
Ratio CO_2: $\frac{\text{pH 7.0}}{\text{pH 4.0}}$				2 07	1.82	

The pronounced accumulation of the carbon dioxide of respiration in the pH 7.0 solutions may be accounted for by the strong tendency of the $CO_3^{=}$ ions or the HCO_3^- ions at this pH to combine with free cations to form harmless soluble carbonates. In the solutions of the lower pH this tendency is less pronounced and the accumulation of the carbon dioxide of respiration in the form of soluble carbonates would be correspondingly lower. It is to be emphasized here that the method of analyses employed in these tests, in which the test samples were acidified to liberate carbon dioxide from solution, would account not only for the free gas in solution but also for all carbonates in solution. Thus, the carbon dioxide content of the culture solutions, in this connection, refers not only to the gas in solution but also to the soluble carbonates formed as a consequence of its presence.

In general, then, it may be said that, as the natural consequence of the respiratory processes, these culture solutions in contact with the roots of vigorously growing plants tended to accumulate carbon dioxide at the same time that they were being depleted of oxygen, the prime factor which determined the degree of this accumulation being the pH of the culture solution.

Further inspection of the data of table 5, shows that the solutions of the non-aerated cultures, at the end of a 6-hour test period contained considerably more carbon dioxide than did the solutions of the aerated cultures, as is indicated by the ratios between the average carbon dioxide content of the non-aerated and the aerated solutions. These ratios show the average carbon dioxide content of the solutions of the non-aerated cultures at pH 4.0 and pH 7.0 to be 1.34 and 1.21 times that of the solutions of the corresponding aerated cultures, respectively. This clearly indicates that, undoubtedly by its sweeping action, the aerating process, which maintained the available oxygen supply of the solutions at a relatively high level against the depleting effects of the absorbing roots, at the same time retarded carbon dioxide accumulation.

However, a careful comparative consideration of all the evidence here produced reveals no significant beneficial or injurious influence of the carbon dioxide content of any of the culture solutions in question, which may be definitely related to growth yields, nitrogen absorption rates, or to the oxygen content of the culture solutions in these investigations.

In conclusion, it is to be assumed, of course, that the relative conditions, with respect to the oxygen and carbon dioxide relations, which prevailed in the culture solutions at any given instant during the growth period of the plants, were at least somewhat similar to those which prevailed at the end of the 6-hour test intervals here considered, but differed from them in degree as determined by the size and vigor of the experimental plants. It is not to be assumed, however, that equilibrium conditions could be attained during an experimental interval of 6-hours, nor is it probable that stable equilibrium could ever be attained during the growth cycle of the experimental plants with respect to absorption rates or with respect to oxygen and carbon dioxide relations in the culture solution employed in these studies. A test interval of 6 hours was arbitrarily chosen and was taken to be of sufficient duration conveniently to establish significant differences in the criteria here considered between the aerated and the non-aerated plants.

SUMMARY

Tomato plants were grown in aerated and non-aerated culture solutions at two pH levels. Analyses were made for oxygen and carbon dioxide content of the culture solutions, and the cation and anion absorption ratios were determined after the roots of the plants had been immersed in the solutions during 6-hour test intervals. The following results were evident:

The aerated culture solutions invariably showed a higher oxygen tension than did the corresponding non-aerated solutions at both pH levels. Hydrogen-ion concentration, as such, had no apparent influence upon the oxygen tension of the solutions.

Aeration produced a marked increase in the rates of absorption of cation, anion, and total nitrogen from the culture solutions, over the corresponding rates from the non-aerated solutions at both pH levels.

The rates of absorption of cation nitrogen from the solutions at pH 7.0 were higher than the corresponding rates at pH 4.0. Conversely, the rates of absorption of anion nitrogen from the solutions at pH 7.0 were lower than the corresponding rates at pH 4.0.

Yields produced by the aerated cultures were approximately double the yields produced by the corresponding non-aerated cultures.

Rates of total nitrogen absorption (cation plus anion nitrogen) were slightly but consistently higher from solutions at pH 7.0 than from the corresponding solutions at pH 4.0.

Growth yields produced by the cultures at pH 7.0 were invariably higher than those produced by the corresponding cultures at pH 4.0, and these paralleled the absorption rates of total nitrogen.

Yields of plant material produced by the aerated cultures were approximately double the yields produced by the corresponding non-aerated cultures.

Accumulation of carbon dioxide occurred in all solutions in contact with plant roots. Accumulation at pH 7.0 greatly exceeded that at pH 4.0. As the result of the sweeping action of the aerating process, accumulation in the non-aerated solutions invariably exceeded that in the corresponding aerated cultures.

Carbon dioxide accumulation in the culture solutions appeared to be without effect upon growth, nitrogen absorption rates, or oxygen content of the solutions.

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PLATE 1

EFFECT OF CONTINUOUS AERATION OF CULTURE SOLUTION ON GROWTH OF TOMATO PLANTS

Representative plants 42 days old grown in culture solutions at pH 7.0, above, and at pH 4.0, below, just before being transferred from 2-liter to 4-liter containers. Non-aerated plants at left, aerated plants at right.

THE DECOMPOSITION OF ORGANIC MATTER IN RELATION TO SOIL FERTILITY IN ARID AND SEMI-ARID REGIONS[1]

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All agricultural soils contain organic matter in varying amounts, depending chiefly on climatic factors and cultural practices. Its physicochemical nature and biological transformation have been the subject of extensive study, especially since the importance of microorganisms to soil fertility and plant growth became realized.

Generally speaking, the agricultural soils of arid and semi-arid regions are characterized by their great deficiency in both organic matter and nitrogen, but the rôle played by the organic fraction in these areas is but poorly understood. The problem has been further complicated by the advent of artificial fertilizers, which marks an altogether new era in agricultural development.

Waksman's recent book (11) is such a comprehensive treatise on the subject of soil organic matter that no attempt will be made here to review the voluminous literature. Most investigational work has been confined to the humid sections; it is the object of this investigation to study the rôle of organic matter in soil fertility of the drier regions. Chief consideration will be given to its influence on the liquid phase of the soil, to rates of decomposition as influenced by different factors, and to chemical changes produced by microbiological activities. Attempts will also be made to introduce the plant as a criterion in this respect.

EXPERIMENTAL PROCEDURE AND RESULTS

The growing plant is preëminently concerned with the soil solution; inasmuch as decomposing organic matter is believed to increase the solubility of

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various plant nutrients, the first set of experiments is an attempt to study the influence of different organic materials on the liquid phase of the soil as indicated by 1:5 water extracts.

Three calcareous soils, Gila fine sandy loam, Gila clay loam, and Pima clay, were chosen from series in the Tucson area. As organic materials were selected fresh cow manure, alfalfa (about half-bloom stage), and mature stalks of hegari sorghum. Duplicate 100-gm. air-dry samples of each soil were weighed into glass tumblers with the following treatments: (*a*) check, no treatment, (*b*) 1 gm. (air-dry and powdered) of each organic material. Moisture was brought to and maintained at the optimum, and the tumblers were incubated at room temperature (approximately 30°C. during summer). After certain intervals of time, treated and control soils were analyzed as follows: The contents of the tumblers were transferred to wide-mouthed bottles with 500 cc. of CO_2-free distilled water, placed in an end-over-end shaking machine, and shaken for one hour. The bottles were then allowed to stand for a few minutes so that the heavier soil particles could settle. Total soluble salts were determined on this supernatant liquor by means of a portable conductivity bridge, and pH measurements were made by means of the hydrogen electrode.

Following this, the suspensions were saturated with CO_2 for 15 minutes and then filtered. Phosphates were determined in the filtrates by the colorimetric molybdenum blue method, and nitrates were determined by the phenol-disulfonic acid method. The pH is expressed on 1:5 extracts; whereas soluble salts (T.S.S.), phosphates (CO_2-soluble PO_4), and nitrates (NO_3) are reported in parts per million on the air-dry soil. Since the results obtained for the three different soils were very similar, only the data for Gila fine sandy loam are given in table 1.

Evidently the various organic materials had no significant influence on the pH, which is in agreement with the findings of Stephenson (7) and others. Calcareous soils are heavily buffered on the alkaline side, hence it is conceivable that even appreciable amounts of CO_2 will have negligible effects on the final reaction.

There are only slight variations in soluble salts in the case of untreated soils; this fact suggests that microbiological activities proceed at a relatively slow rate. If organic matter is added, however, considerable changes are produced. Manure invariably caused a decrease during the first few weeks, to be followed by a gradual and continuous increase. In the case of alfalfa, the lag period, if any, is relatively short, the soluble salts increasing rather rapidly, in close correlation with increases in nitrates. Hegari showed a marked reduction in all cases; this fact can be explained by the assimilation of nitrates and their subsequent conversion to microbial protoplasm.

With respect to nitrates, it will be noticed that there is a gradual increase in this ion in untreated soils, pointing to the fact that complex organic compounds are being slowly mineralized. It is a remarkable fact that manure with a relatively high nitrogen content (1.90 per cent) should show a lag with respect

TABLE 1

Effect of organic matter on aqueous (1:5) extracts of gila fine sandy loam

TIME	pH	T.S.S.	PO_4(CO_2-SOL.)	NO_3
weeks		*p.p.m.*	*p.p.m.*	*p.p.m.*
Check, no treatment				
0	8.60	375 0	13.50	50 6
1	8.69	757 5	13 50	49.3
2	8 67	605.0	14 00	54.6
3	8.73	580.0	15.60	83.7
5	8.71	455 0	13 20	138.5
8	8 56	680 0	11 50	116 5
12	8 61	617 5	15 60	180.0
20	8 75	605 0	20 00	240 0
Plus 1 per cent manure				
0	8 68	632 5	53 0	72 0
1	8 59	637 5	61 5	14.0
2	8 68	560 0	59 7	10 1
3	8 71	570 0	59.0	24.0
5	8 71	570 2	57 5	81 8
8	8 52	740.0	54 0	120.0
12	8 59	795 5	72 5	257.0
20	8 60	920 1	90 0	360 1
Plus 1 per cent alfalfa				
0	8.23	1130.0	9.25	36 0
1	8 49	950.0	10 44	75.0
2	8 38	1220 0	11.88	313.0
3	8 38	1222 5	13 60	400.1
5	8 43	1330 0	12 80	514.3
8	8 32	1505.5	12.50	600 0
12	8 42	1635 0	15.0	719 4
20	8.44	1655 0	24 8	900.0
Plus 1 per cent hegari				
0	8 44	915 0	15.00	48.6
1	8 64	592.5	17 32	8.8
2	8.71	575.0	17.50	7.0
3	8.80	602.5	16.00	0.0
5	8.78	550.0	15.51	0 0
8	8.72	620 0	14 00	0 0
12	8 78	590.0	18 50	0.0
20	8.70	580 0	22 50	1.5

to nitrification. Evidently, the nitrogen is tied up in a more or less resistant form, which must be regarded as a distinct advantage. Alfalfa, on the other hand, is nitrified very rapidly, whereas hegari conforms to the well-known principle of nitrate assimilation by microorganisms in the presence of excessive amounts of carbohydrate material.

In all these studies, the application of manure markedly increased the amount of CO_2-soluble phosphates, even without any decomposition, whereas alfalfa and hegari had almost negligible effects when compared with the untreated soils. This suggests that increases in phosphates came from their addition with the different materials and not from the mineral fraction of the soil as a result of microbiological activities. Breazeale and Burgess (2) have shown that in the presence of $CaCO_3$, CO_2 has no appreciable effect on the solubility of phosphates. Furthermore, inasmuch as phosphate availability is largely a function of pH, these results are in agreement with the fact that the different organic materials had no significant influence on the pH.

Percolation studies

It was felt that the use of 1:5 extracts might not be a sufficiently sensitive method of measuring changes in the liquid phase of the soil as a result of microbiological activities. Hence percolation studies were resorted to, Gila fine sandy loam being selected for this purpose. Duplicate 1500-gm. air-dry soil samples were placed in large percolation tubes with the same treatments as outlined for the 1:5 extracts. The soils were then leached with consecutive 1000-cc. volumes of CO_2-free distilled water after certain intervals of time, and the percolates were analyzed for soluble salts (bridge method), pH (hydrogen electrode), calcium and magnesium (soap titration method), potassium (colorimetric cobalti-nitrite method), bicarbonates (by titrating against $0.02N$ H_2SO_4, using methyl orange as indicator), and nitrates (phenol-disulfonic acid method). The results are recorded in table 2.

Tremendous increases in soluble salts, bicarbonates, and calcium are obtained in cases where organic matter was added, especially in the first percolation, which took only 12 hours. The increases are greatest for alfalfa, followed by hegari and manure, respectively. It will be shown later that this is the order in which these three materials decompose. From the data it appears that after 6 weeks had elapsed, the organic matter was incapable of supplying any more plant nutrients to the liquid phase than in the case of untreated soils.

These results are also interesting from the standpoint of the fundamental differences between humid and arid regions. Whereas in the former, the soil solution is being constantly depleted as a result of leaching, in the latter such losses are negligible. This being the case, it is conceivable that CO_2 liberated by microbial activities will not play the same significant rôle as in the case of humid regions; in fact, we will show later that the bulk is returned rapidly to the atmosphere without seriously affecting the chemical equilibrium of the soil.

Rates of decomposition of organic materials

The data obtained with percolation studies suggested rapid decomposition of organic matter under these experimental conditions. Hence quantitative studies on rates of decomposition as measured by CO_2 production were undertaken.

Triplicate 100-gm. air-dry samples of Gila fine sandy loam were placed in pint milk bottles with the same treatments as outlined previously. The moisture content was brought to the optimum, and the flasks were closed with stoppers fitted with tubes for aspiration and incubated at 30°C. At the stated times the flasks were connected in an aeration unit of the type described by Waksman and Fred (12). The CO_2 produced from the different samples was determined volumetrically by precipitation with excess 0.2N $Ba(OH)_2$, the excess base being titrated rapidly against 0.2N oxalic acid in the presence of phenolphthalein as indicator. The results obtained are given in figure 1.

TABLE 2

Percolation studies—effect of organic matter on soluble salts, pH, calcium, magnesium, potassium, bicarbonates, and nitrates

(Gila fine sandy loam)

TREATMENT		CHECK, NO TREATMENT	1 PER CENT MANURE	1 PER CENT ALFALFA	1 PER CENT HEGARI	CHECK, NO TREATMENT	1 PER CENT MANURE	1 PER CENT ALFALFA	1 PER CENT HEGARI
		12 hours				1 week			
T.S.S.	*p.p.m.*	574.0	1516.0	2470.0	2019.0	361 0	1128.0	1630 0	2236.0
pH		8 42	8.30	7.48	7 80	9.03	8.92	8.22	8.12
Ca	*p.p.m.*	135	375.0	840 0	660.0	82 5	262.0	660.4	435.0
Mg	*p.p m.*	11.0	19 0	37.1	37.0	7.5	11.5	15.0	15.0
K	*p.p.m.*	44.0	80 0	213 3	128.5	23.0	83.6	307.7	30.7
HCO_3	*p.p.m.*	634	976.0	1464 0	1220.0	402.0	1073.0	2488.0	1464.0
NO_3	*p p.m.*	203.8*	293.0*	886 0*	318.9*	59.6	59.6	70.6	57.2
		6 weeks				12 weeks			
T.S.S.	*p.p.m.*	415.0	240 0	570.5	273.0	273.0	203.3	485.0	216.5
pH		8 50	8.0	8 10	7.90	7.88	7.46	7.5	7.5
Ca	*p.p.m.*	102.6	72.0	123.0	57.0	69.0	45.0	152.0	48.0
Mg	*p.p.m.*	1.50	2.1	3.0	4.5	4 5	4.5	4.5	3.0
K	*p.p.m.*	6.0	13.3	23.0	32.0	11.7	17.4	33.3	24.3
HCO_3	*p.p.m.*	195.2	165.8	122.0	185.4	117.2	80.5	80 5	104.9
NO_3	*p.p.m.*	92.4	22.0	283.8	15.4	46.9	24 0	176.0	tr.

* Includes ammonia-nitrogen.

A study of the data obtained shows that large quantities of CO_2 were liberated where organic matter was added, the order being alfalfa, hegari, and manure, respectively. In all cases, maximum production was reached at the end of the first or second day, the quantities decreasing rather rapidly after the

third day, with the exception of the sorghum, which maintained a fairly steady rate after the fourth day. These results are in agreement with those of other investigators, but larger amounts of CO_2 were produced in our studies, especially during the first few days. Attention is called to the fact that after one month had elapsed, the differences between treated and control soils were almost negligible.

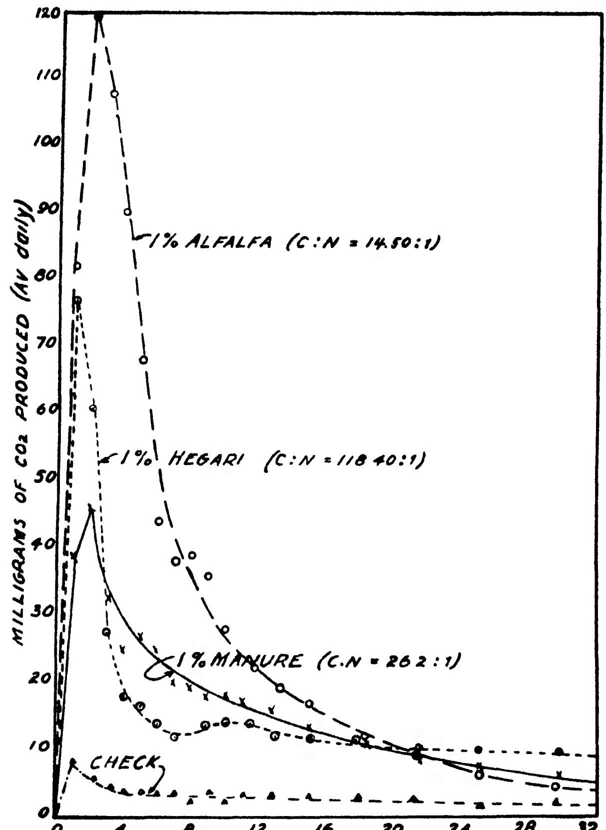

FIG. 1. RATE OF DECOMPOSITION OF ALFALFA, HEGARI, AND MANURE IN THE SOIL AS MEASURED BY CO_2 PRODUCTION

From these results it also appears that the C:N ratio is not the only factor deciding rates of decomposition, since manure, having a ratio of 26:1, decomposes more slowly than hegari sorghum with a ratio of 118:1. This is in agreement with the findings of Hutchings and Martin (3). The relatively slow rate at which manure decomposes is regarded as a distinct advantage.

Decomposition of "Pure" organic compounds in the soil

Natural organic materials consist of a great many chemical complexes such as sugars, hemicelluloses, and lignins. A study of the relative rates at which

some of the more abundant complexes decompose in the absence of others is regarded as important in order to understand their rôle in the formation of soil humus. Although it is realized that their relative rates of decomposition as "chemically pure" substances are no precise index as to their behavior when tied up in complex combinations in plants or manures, yet such data are regarded as important in the present investigation.

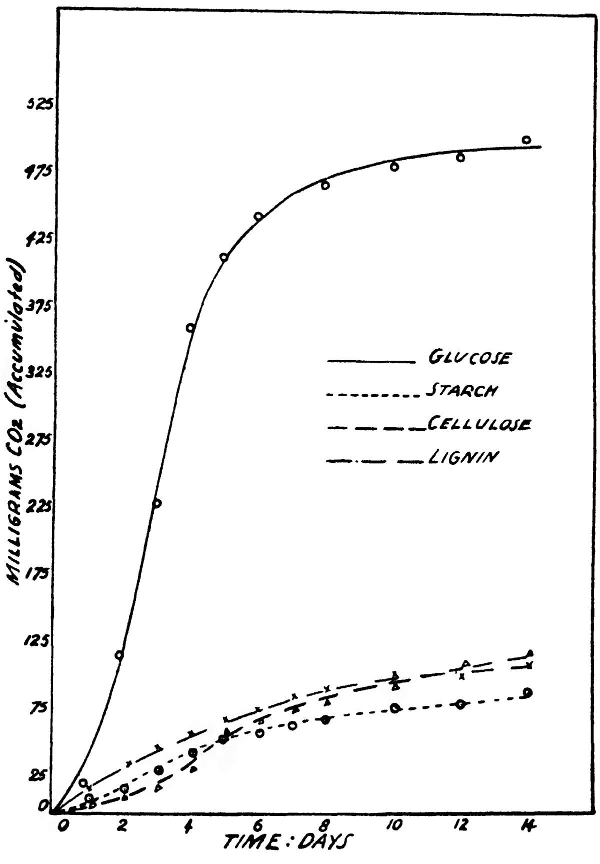

FIG. 2. RATE OF DECOMPOSITION OF GLUCOSE, STARCH, CELLULOSE, AND LIGNIN IN THE SOIL AS MEASURED BY CO_2 PRODUCTION

Duplicate 50-gm. air-dry samples of Gila fine sandy loam were placed in pint milk bottles with 1 per cent applications of glucose, starch, cellulose (finely macerated filter paper), and lignin (prepared from corn cobs [5]). Moisture was brought to the optimum, the flasks were closed with an aspiration outfit and incubated at 30°C., and the CO_2 liberated was determined as described previously. The results are given in figure 2.

If CO_2 production is taken as an index of rates of decomposition, then the order in which these substances undergo decomposition is glucose, lignin, cellulose, and starch. These data are in agreement with the findings of Mc-

George and Greene (4). Smith and Brown (6) have also shown that certain soil fungi are capable of decomposing lignins almost as fast as xylans or cellulose.

It is realized that, inasmuch as the compounds studied are essentially nitrogen-free, their relative rates of decomposition will be greatly affected by the nitrate balance of the soil. Furthermore, glucose, and possibly starch, too, may be utilized as sources of energy by organisms like Azotobacter, whereas cellulose and lignins are believed to be unavailable to such organisms. Finally, Waksman (11) has shown that the lignins are tied up in complex combinations with the proteins in humus. This "ligno-protein" complex does, however, undergo slow decomposition by microörganisms.

Influence of moisture on the decomposition of organic matter

Moisture is probably the most important single limiting factor affecting the agricultural development of arid and semi-arid regions. This applies not only to final crop yields, but also to various physicochemical and biological soil properties, the latter being associated with microbial activity and humus balance. Consequently a study of the decomposition of organic matter at different moisture contents is of fundamental importance in the present investigation, and CO_2 evolution appeared to be the most sensitive method for such studies.

The experimental technique consisted of placing 50-gm. air-dry samples of Gila fine sandy loam in pint milk bottles with 1 per cent applications of powdered alfalfa used in previous studies. The following moisture contents, expressed as percentage of the water-holding capacity, viz. 35 per cent, were selected: air-dry (3.06), 15, 35, 50, 75, and 100. Up to 50 per cent, the samples were thoroughly mixed after the required amount of water had been added. The flasks were then closed with attachments for aspiration and incubated at 30°C., and the CO_2 was determined as described previously.

In order to maintain the different moisture contents, the air, after being washed with 40 per cent KOH solution to remove CO_2, was passed slowly through different concentrations of H_2SO_4 prepared according to the vapor pressure data of Wilson (16) and of Thomas (9). At the close of the experiment, moisture determinations were made on each sample. It was found that the different percentages had been satisfactorily maintained within 2 per cent. The results obtained in this investigation are given in figure 3.

From the data it is evident that negligible quantities of CO_2 are evolved from the air-dry soil. Beyond this, however, relatively small additions of moisture give rise to large amounts of CO_2, the quantities increasing up to almost complete saturation. At the optimum and toward saturation, maximum production always occurs during the first or second day, but there is a delayed maximum at the lower moisture contents with the result that relative rates are kept up at a higher level in the latter case. When total quantities after 19 days are considered, there is found a progressive increase in CO_2 production from 15 per cent of the water-holding capacity up to complete

saturation, although the differences are not very great. This is in agreement with the results of Waksman and Gerretson (13).

One factor of great importance is the relatively large amounts of CO_2 liberated at 15 per cent of the water-holding capacity, which value is considerably less than the wilting coefficient as calculated from the moisture equivalent by use of the empirical factor 1.84. This fact suggests that the microflora, during its era of adaptation, has shifted the cardinal points in its life processes to the

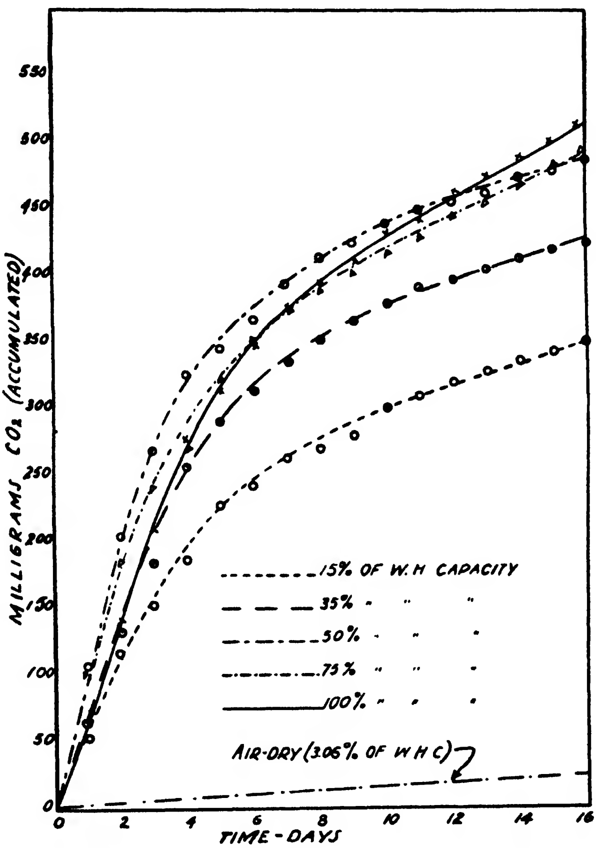

FIG. 3. INFLUENCE OF MOISTURE ON THE DECOMPOSITION OF ALFALFA IN THE SOIL AS MEASURED BY CO_2 PRODUCTION

left with respect to moisture; i.e., we are dealing essentially with a semi-xerophytic flora. At the same time, however, the specific groups of organisms appear to be capable of functioning very efficiently over a wide range of moisture contents.

Influence of temperature on the decomposition of organic matter

Next to moisture, and in some cases perhaps of even greater biological significance, is the temperature factor, these two being the chief climatic

agencies determining plant distribution and soil fertility in general. Wollny (17), Waksman and Gerretson (13), and others have shown that the rate at which organic matter decomposes increases with increasing temperature, with an optimum of around 32°C. as found by CO_2 production from soils. No data seem to be available with respect to the arid regions; hence a study was undertaken on the effect of temperature on the decomposition of organic matter as measured by CO_2 production.

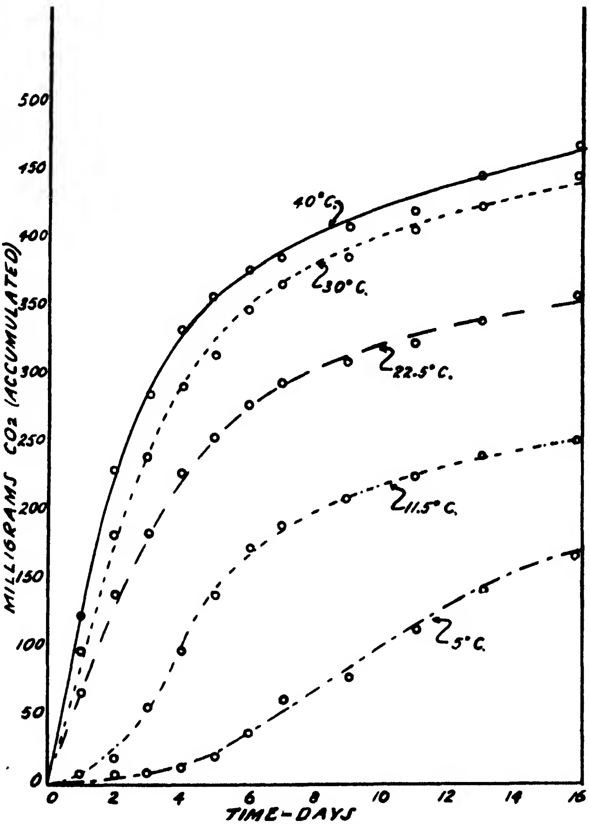

FIG. 4. INFLUENCE OF TEMPERATURE ON THE DECOMPOSITION OF ALFALFA IN THE SOIL AS MEASURED BY CO_2 PRODUCTION

The experimental procedure was the same as that outlined in the previous experiment, with the exception that the moisture was adjusted at the optimum and the flasks were incubated at 5°C., 11.5°C., approximately 22.5°C. (room temperature), 30°C., and 40°C. The CO_2 produced was again determined as described previously. The results are given in figure 4.

There is a progressive increase in CO_2 production with increasing temperature, the optimum lying somewhat above 40°C. Only small amounts are produced at 5° and 11.5°C. during the first few days, but the quantities gradually

increase, the optimum being delayed as much as one week. From room temperature up to 40°C. the largest amounts are produced during the first and second days.

From the figure it is also evident that temperature is a more important factor than moisture, but that microörganisms are capable of adaptation, hence able to liberate CO_2 over a wide range of temperature conditions. The fact that optimum production of CO_2 occurs somewhat above 40°C. suggests that, in the case of temperature, the cardinal points have been shifted to the right, approximating a semi-thermophilic microflora.

Fractionations of organic matter at different stages of decomposition

A considerable amount of work has been done with respect to chemical changes in organic matter as a result of microbiological activities. Since most of the researches have been confined to humid regions, considerable time and effort were spent in the present study in an endeavor to investigate this phase of the problem in arid regions.

The experimental technique follows: Duplicate 15-gm. air-dry samples of finely ground manure, alfalfa, and hegari (the same as used in previous studies) were weighed into small Erlenmeyer flasks, inoculated, and brought to the optimum moisture content (approximately 200 per cent) with a 1 per cent suspension of Gila fine sandy loam; the flasks were incubated at 30°C.; and the moisture was kept at the optimum. After certain intervals of time, fractionations were made on the residual material according to the method outlined by Waksman and Stevens (14). Total organic matter is designated as the residual material obtained by weighing after the required intervals of time had elapsed. The results obtained are given in figures 5, 6, and 7.

There is a rapid destruction of organic matter, the relative rates of decomposition being in agreement with the evolution of CO_2 previously reported. Evidently, as has already been pointed out, the C:N ratio is not the only deciding factor in respect to rapidity of decomposition, since hegari (C:N = 118:1) decomposes more rapidly than manure (C:N = 28:1). Presumably, the nitrogen in the latter case is tied up in a rather resistant form, which must be regarded as a distinct advantage. Hutchings and Martin (3) have shown that the chemical composition of the different complexes is an important consideration: in our studies the rates of decomposition are positively correlated with the amount of water-soluble material. As was found with CO_2 measurements, decomposition processes are extremely rapid during the first few weeks, after which time they slow down rather abruptly.

The water-soluble fraction shows an interesting phenomenon: whereas there is an appreciable decrease in this constituent, especially in the case of alfalfa and hegari during the first two weeks, the amounts are in all cases kept up at a rather high level. This is in distinct contrast with results reported by Waksman and Tenney (15), where this fraction usually disappears very rapidly. An explanation for our results is to be found in active decomposition of hemi-

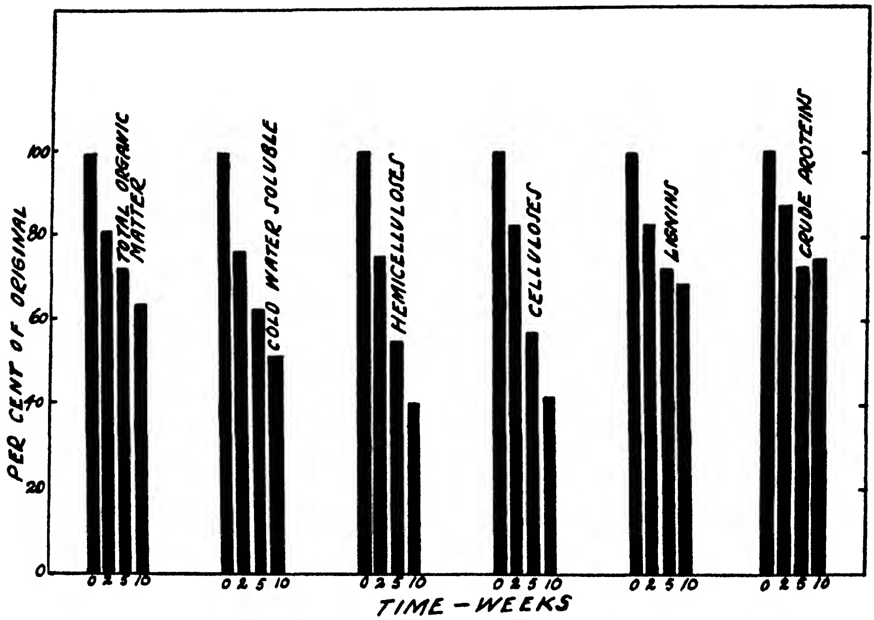

FIG. 5. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF MANURE WHEN INOCULATED WITH A 1 PER CENT SUSPENSION OF GILA FINE SANDY LOAM

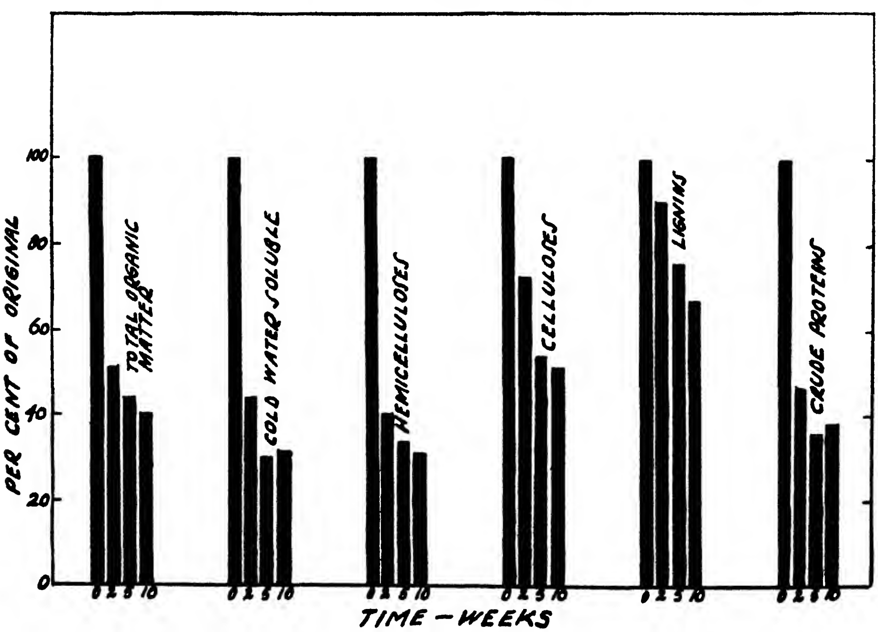

FIG. 6. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF ALFALFA WHEN INOCULATED WITH A 1 PER CENT SUSPENSION OF GILA FINE SANDY LOAM

cellulose, cellulose, proteins, and even the lignins. In humid regions the more resistant fractions like the lignins tend to accumulate, according to Waksman and Tenney (15); our studies indicate that such is not the case.

The protein fraction likewise undergoes fairly rapid decomposition in the case of alfalfa and manure, whereas the increases found in the case of the sorghum are in part due to nitrate assimilation as well as to possible fixation by organisms like Azotobacter. In general, one might say that the trends of decomposition of the different fractions are substantially the same as those reported by other investigators (15), although it is evident that in our studies decomposition processes are carried on more rapidly and that the more resistant

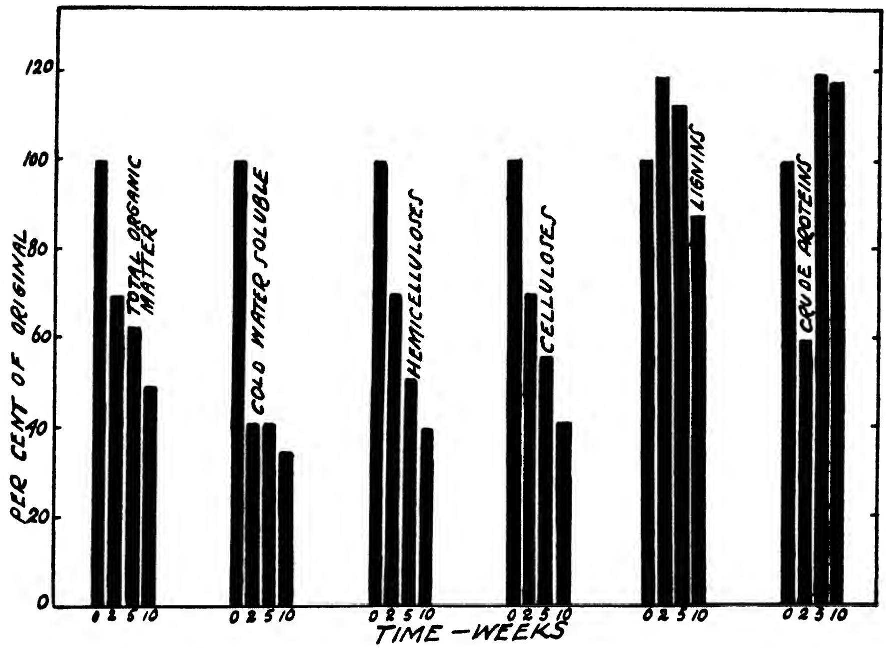

FIG. 7. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF HEGARI SORGHUM WHEN INOCULATED WITH A 1 PER CENT SUSPENSION OF GILA FINE SANDY LOAM

fractions, notably the lignins, are also relatively rapidly attacked. This fact, no doubt, accounts in part for the fundamental difference between arid and humid regions as far as accumulation of organic matter is concerned.

Fractionation of organic matter in the presence of soils

Waksman's excellent work (11) on the fractionation of organic matter at different stages of decomposition provides a method whereby such analyses may be carried out in soils, but there are several obstacles in the way, some of a purely mechanical nature, but others, like the presence of relatively large amounts of carbonates and ferric iron, of a rather serious chemical nature. Waksman suggests removal of iron and aluminum as the hydroxides before

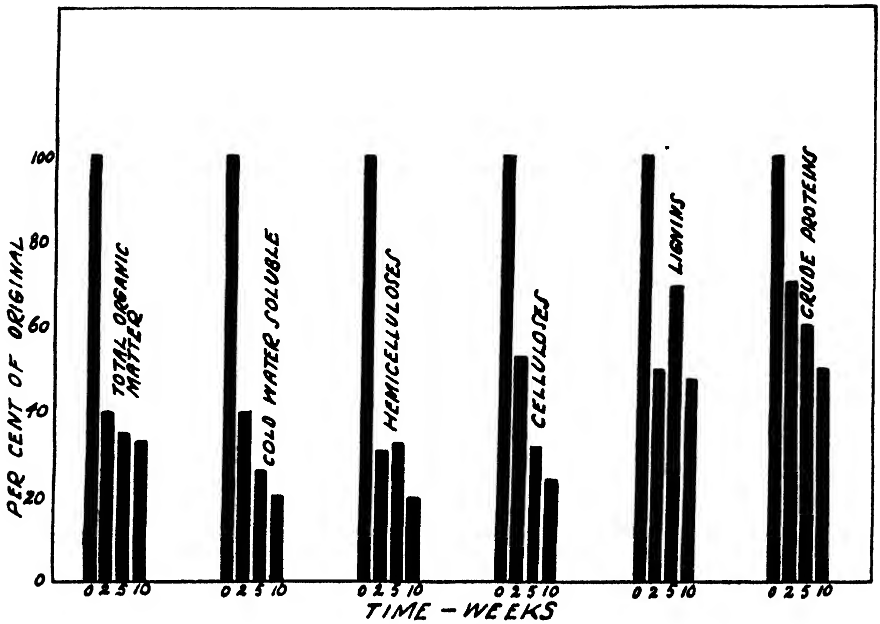

FIG. 8. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF ALFALFA IN THE PRESENCE OF GILA FINE SANDY LOAM

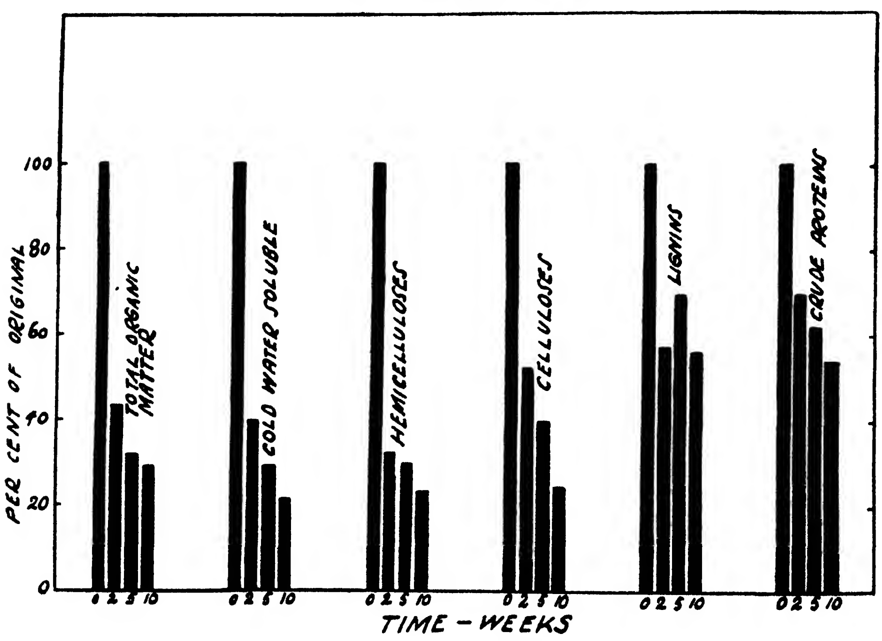

FIG. 9. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS OF ALFALFA IN THE PRESENCE OF TUBAC CLAY LOAM

analyzing the acid extracts for reducing sugars but evidently does not allow for oxidation of the sugars during hydrolysis. Naturally, the final object is to develop methods that would enable us to follow the fate of the different organic constituents in actual presence of the soil in order to understand the various transformations that occur under field conditions. Considerable time and effort were therefore spent in this investigation in an endeavor to overcome some of the major difficulties already referred to.

Two soils were selected, viz., Gila fine sandy loam (9.25 per cent $CaCO_3$) and Tubac clay loam, containing no $CaCO_3$. Duplicate 30-gm. air-dry samples of each soil were weighed into glass tumblers, 10 gm. of finely ground alfalfa (about half-bloom stage, but not the same sample as used previously) was added, the moisture was brought to and kept at the optimum, and the tumblers were incubated at 30°C. After certain intervals of time, fractionations were made according to the method of Waksman and Stevens (11).

Reference was made previously to the fact that ferric iron interferes with the determination of reducing sugars. Furthermore, there seems to be no suitable method whereby it is possible to separate the iron or to render it harmless as far as oxidation of the sugars during hydrolysis is concerned. However, it is a well-known fact that the arid soils of this region contain relatively small amounts of soluble iron, even when treated with concentrated acids. Iron determinations were made on the two soils (*a*) by boiling with 2 per cent HCl for one hour, (*b*) with 80 per cent H_2SO_4 in the cold followed by dilution and prolonged boiling. In no case did more than 1 per cent iron become soluble. Further investigations showed that these amounts of iron are too small to interfere with the determination of reducing sugars in estimating the hemicellulose and cellulose fractions. No attempts were therefore made to separate the iron before analyzing for reducing sugars. The results on these fractionations are given in figures 8 and 9.

A study of the data seems to indicate that decomposition processes follow the same general trends in both soils, suggesting that $CaCO_3$ had no marked influence in this particular case. Furthermore, although the sample of alfalfa used was not the same as that in previous investigations in the absence of soil, it appears that the trends of decomposition were essentially the same in both cases. The hemicellulose and cellulose fractions disappear somewhat more rapidly in the presence of soil, whereas the protein complex is maintained at a higher level. The lignins show some variability in both soils.

Neubauer studies

The plant is the final criterion in all investigational work dealing with soil fertility. The Neubauer method, therefore, suggested itself as the most suitable for such studies.

Duplicate 100-gm. samples of air-dry Gila fine sandy loam were placed in enamelled dishes with 1 and 5 per cent applications each of alfalfa, manure, and hegari. In order to study the effect of different stages of decomposition

on the availability of phosphorus and potassium, four series were prepared, the first being started in summer (July), whereas the other three were spread over a range of about five months. Moisture was in all cases maintained at the optimum, and the samples were incubated at room temperature.

TABLE 3

Effect of organic matter on availability of phosphates and potassium—P_2O_5 and K absorbed by rye seedlings in Neubauer studies

(Gila fine sandy loam)

TREATMENT	TIME OF DECOMPOSITION	GERMINATION	P_2O_5 ADDED WITH O.M.	P_2O_5 ABSORBED	K ADDED WITH O.M.	K ABSORBED
	weeks	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Sand blank (ave. 6)	0	88	0	29.24	0	13 00
Check, no treatment	0	77	0	31.75	0	25.01
1 per cent manure	0	88	17 20	38.10	4 0	24 85
5 per cent manure	0	89	86.00	36 70	20 0	28 00
1 per cent alfalfa	0	71	5 60	27 80	0 8	18 80
5 per cent alfalfa	0	77	28 00	29.10	4 0	26.00
1 per cent hegari	0	76	2.50	31.50	0.9	21.50
5 per cent hegari	0	84	12.50	29.60	4 5	23.80
Check, no treatment	4	65	0	28 60	0	18 80
1 per cent manure	4	66	17 20	32 00	4 0	24 30
5 per cent manure	4	79	86 00	40 20	20.0	28.40
1 per cent alfalfa	4	85	5.60	32 60	0.80	28.50
5 per cent alfalfa	4	90	28 00	35.88	4 00	36.10
1 per cent hegari	4	61	2.50	26 40	0 90	20 10
5 per cent hegari	4	91	12.50	35 25	4 50	32 90
Check, no treatment	11	75	0	33 80	0	20.00
1 per cent manure	11	88	17.20	43.15	4.0	24 50
5 per cent manure	11	87	86.00	41.75	20.0	28 10
1 per cent alfalfa	11	86	5 60	35.00	0 80	26 00
5 per cent alfalfa	11	84	28.00	41.10	4 00	28 90
1 per cent hegari	11	83	2.50	30.70	0 90	28 50
5 per cent hegari	11	92	12.50	38.50	4.50	36.80
Check, no treatment	20	56	0	28.00	0	20.00
1 per cent manure	20	78	17.20	39.70	4.00	28.00
5 per cent manure	20	78	86.00	47.50	20.00	34.50
1 per cent alfalfa	20	85	5.60	29.70	0.80	28.75
5 per cent alfalfa	20	88	28.00	38.20	4.00	41.40
1 per cent hegari	20	91	2.50	37.20	0.90	28.40
5 per cent hegari	20	95	12.50	35.00	4.50	38 40

During November all four series were simultaneously subjected to the Neubauer method, rye seedlings being used according to the standard technique. The plants were harvested 18 days after germination and analyzed for phosphates by the volumetric molybdate method and for potassium by the colorimetric cobalti-nitrite method. The results are given in table 3.

Unfortunately, the average percentage germination was very poor, although we feel that the results are still significant. It will be noticed that the soil in question is deficient in phosphates and that the organic matter, excepting in cases where no time was allowed for decomposition, invariably increased the amount of phosphates absorbed in the order manure, alfalfa, and hegari, respectively.

Phosphate determinations were made on the three organic materials; it will be noticed that increased absorption by the seedlings follows the amounts added rather closely. Furthermore, with the exception of the 20-week period in the case of 1 per cent hegari, the amount of P_2O_5 added with the different organic materials and that absorbed from the untreated soils are in excess of that absorbed from the treated soils. This presents a problem: did any phosphates come from the soil during microbiological processes or are the increases due solely to the phosphates added with the organic materials? Results obtained from 1:5 extracts showed that decomposing organic matter has negligible effects on the mineral phosphate content of the soil, and the Neubauer results seem to confirm this. Palpably, unless the increases obtained are in excess of the plant foods added, one is not justified in concluding that the organic matter had caused any increases in phosphate availability from the mineral fraction of the soil. Furthermore, the same and, no doubt, greater increases could have been obtained by the direct application of water-soluble phosphates.

The situation appears to be different with respect to absorption of potassium. In all cases, more particularly with the heavier applications and longer incubation periods, the organic matter markedly increased the availability of potassium to plants. In the case of all 1 per cent applications, the increases are in excess of the amount of potassium added with the organic matter, indicating that some potassium must have come from the inorganic fraction of the soil. Furthermore, increased absorption is positively correlated with the relative rates of decomposition of the different organic materials, indicating that the process is essentially biological in nature. It is believed that the increased solubility is brought about by way of base exchange due to interaction between carbonic acid and the potassium present in the zeolitic complex.

DISCUSSION

We may now discuss the general problem of organic matter decomposition in arid and semi-arid soils in the light of the foregoing experimental results and correlate them, if possible, with those of other investigators.

Organic matter is a natural constituent of all agricultural soils and hence is intimately associated with the numerous factors that have influenced the soil in the past and which determine its present state of fertility. Itself of complex nature, consisting of numerous types of organic compounds, it enters into physical, chemical, and biological reactions, some of which are difficult, if not impossible, to study satisfactorily. The fact that it is the home of countless

microörganisms completes a complicated picture, which has through the ages interested thoughtful men.

Its origin, chemical composition, and importance in nature have been recently well reviewed by Waksman (11), who is one of the most active workers in this field. The fundamental importance of microörganisms in keeping plant food elements in continuous circulation is probably the most important factor to be realized. But one must bear in mind that the advent of artificial fertilizers has in many respects upset the older concepts of soil fertility, a fact which is not always sufficiently well appreciated.

Scientific soil research realizes the fundamental importance of climatic conditions. Generally speaking, the soils of arid regions are characterized by their relative stability and constructive genesis, which are due chiefly to relatively porous structure as a result of predominantly physical weathering and a high degree of base saturation, chiefly with calcium. Physically then, with the exception of the black alkali lands, these soils offer much less difficulty in cultivation than do those of humid regions, where weathering agencies are chiefly chemical and destructive in nature. This being the case, it is obvious that organic matter will have totally different effects in the two regions. In the first place, humid soils contain larger total amounts due to climatic factors and plant development. Secondly, whereas a satisfactory humus balance is regarded as essential for both the physical condition and the fertility of humid soils, such does not appear to be the case for arid sections, according to the results of Theron and van Wyk (8) and Thorne (10). Finally, the nitrogen problem differs fundamentally in the two regions: humid soils are subjected to constant losses through leaching and abundant plant growth, whereas the arid regions, although generally regarded as deficient in nitrogen, seem to maintain a satisfactory balance with the assistance of active nitrogen fixation.

No physical investigations of the problem were undertaken since Alway and Neller (1), Thorne (10), and others have shown that ordinary applications of organic matter have negligible effects on the physical properties of arid soils. The chemical aspects were regarded as more important, and it was found in the present study that decomposing organic matter had no significant influence on either the pH or phosphate availability of arid calcareous soils, probably the two most acute problems in the fertility of arid soils. Of equally great significance is the fact that its influence on the content of soluble salts depends directly on its rapidity of nitrification, which leads to a very important conclusion, viz., that a very profuse liberation of CO_2 from organic matter is unable seriously to affect the highly buffered and stable system under consideration. This leads us to believe that CO_2 of microbial origin is for the greater part returned rapidly to the atmosphere, contributing but little, if anything, to the maintenance of a satisfactory supply of nutrient ions in the liquid phase. In this connection, McGeorge and Greene (4) have recently shown that sulfur may well be regarded as a suitable substitute for organic matter in arid calcareous soils, because of its beneficial effects on the pH, phosphate availability, and physical condition of these soils.

Extensive studies were undertaken on the decomposition of various organic materials, in view of the fact that few data are available for arid regions. When CO_2 production is taken as an index to rates of decomposition, it is found that, in general, the rates are higher than those reported for humid regions. Investigations on the influence of temperature and moisture on the decomposition of organic matter suggest that the former is a more important factor than the latter, but that microörganisms can adapt themselves over a relatively wide range of conditions. It was also pointed out that the particular flora under question approximates a semi-thermophilic-xerophytic one, accounting in part for the rapidity with which organic matter disappears from arid soils.

Detailed studies on the fractionation of different organic materials both in the presence and in the absence of soil again pointed to rapid decomposition processes. In general, it was found that the trends of decomposition are the same as those reported for humid regions, but there seems to be a more rapid destruction of organic matter as a whole, as well as of some of the more resistant fractions, notably the lignins. Two explanations are advanced for this fact: (*a*) a very active microflora, characteristically high in spore-forming bacteria and actinomyces, may be present and (*b*) the lignin fraction may be less stable under alkaline than under acid soil conditions. Whatever the underlying causes, it is obvious that, because of rapid decomposition processes, it is difficult, if not impossible, to build up the supply of organic matter under these conditions. It appears, therefore, that the value of organic matter in arid regions will depend chiefly on its fertilizing value and its influence on the physical condition of the soil.

SUMMARY

Aqueous 1:5 extracts on three calcareous soils using three types organic matter brought out the following facts:

Decomposing organic matter has no significant influence on the pH of calcareous soils.

Over and above its own phosphorus content, organic matter does not affect the availability of phosphates to any appreciable extent.

The influence of organic matter on soluble salts and nitrates depends primarily on its chemical nature, especially its C:N ratio. The slow rate at which manure is nitrified is regarded as a distinct advantage.

Percolation studies led to the conclusion that during the first few weeks active decomposition results in large increases of soluble salts, especially calcium bicarbonate. After six weeks had elapsed, however, the difference between treated and untreated soils was negligible.

Alfalfa, hegari, and manure decompose in the order mentioned when CO_2 production from soils is taken as an index to rates of decomposition. Larger amounts of CO_2 are produced than are reported for humid regions, and maximum production always occurs during the first or second day.

Glucose, lignin, cellulose, and starch undergo decomposition in the order mentioned when incorporated with a soil and when rates of decomposition are

estimated by CO_2 evolution. Explanations are suggested for the relatively rapid rate at which lignin decomposes, and attention is directed to the significance and importance of this phenomenon.

The rate at which organic matter decomposes increases with increasing moisture up to almost complete saturation. However, considerable losses occur even below the wilting coefficient, and it is suggested that the microbial flora has shifted to the left, the cardinal points with respect to moisture, approaching a xerophytic flora.

Decomposition of organic matter increases with temperature, the maximum being around 45°C. as found by CO_2 production from soils. Although microorganisms are sensitive to low temperatures, adaptations are possible, allowing them to function over a relatively wide range of temperature conditions. It is suggested that in the case of temperature, the cardinal points have been shifted to the right, approximating a thermophilic flora. It appears that temperature is a more important factor than moisture in the decomposition of organic matter, but obviously a combination of the two is the actual criterion.

The bulk of the CO_2 produced by microbial action does not remain in the soil, but is returned rapidly to the atmosphere, without seriously affecting the relatively stable conditions existing.

Fractionation of organic materials at different stages of decomposition shows that in general the trend is the same as that reported by other investigators. But it appears that there is a more rapid disappearance, that the water-soluble fraction is maintained at a higher level, and that the lignin and protein complexes suffer appreciable losses by microörganisms.

In the presence of soil, organic matter undergoes essentially the same trend of transformation, with the exception that the carbohydrate fraction tends to disappear somewhat more rapidly. This results in a more pronounced relative accumulation of lignin and proteins.

Neubauer investigations showed that actively decomposing organic matter is injurious to the growth of seedlings. It further appears that organic matter affects the availability of phosphates only to the extent of its own content of phosphate but markedly increases the availability of potassium from the soil.

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ON THE FORMATION OF STRUCTURE IN SOIL: I. THE STRUCTURE OF SOIL COLLOIDS

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The ability of molecules to associate into groups, forming phases typical of a mesomorphous system, is characteristic of the aggregate condition of soil colloids.

The nematic phase (according to Friedel) is readily observed in two variants —the filamentous and the granular—in concentrated argillaceous suspensions or clay gels in a greatly swollen condition. The smectic phase is formed from water suspensions of clays under the influence of the surface tension film when the suspensions are drying up. The particles of clay are distributed in layers lying at equal distances. In clays, both phases are always anisotropic and possess a considerable double refraction. Humates, isolated from soil, also form a mesomorphous system, but with a lesser double refraction than that of clays. Their smectic phase is identical with that of clay. In some humates, as, for example, in the products of autolysis of the fungus *Aspergillus niger*, a smectic phase with a remarkably strong double refraction is precipitated. Fine quartz sand, mixed with water, after drying is distributed in rows resembling, in their relation to polarized light, the "mica system of Reusch." The phenomenon of twinning is observed not only in sand but also in the nematic and smectic phases of clays and humates and apparently it may serve for the explanation of the distortions frequently observed during the determination of the physico-mechanical properties of soils.

In many cases the behavior of colloids isolated from soil cannot be accounted for by the nature of the absorbed cations. To the most remarkable examples of such anomalous behavior belongs the instability of the suspensions of highly hydrated monovalent clays, e.g., Na-clays. In spite of the high value of the ζ-potential and their high degree of dissociation, the Na-clay sols have the ability to coagulate spontaneously even in low concentrations. It must be noted that in the latter case we are dealing not with common coagulation, i.e., with an irregular accumulation of molecules in the precipitate, but with a "directed coagulation" (35), conditioning optical anisotropy.

The present report is a communication on the spontaneous structures arising in soil colloid sols as a result of a definite distribution of molecules in groups, and on structures arising as a consequence of surface tension (22).

First of all, the ability of Na-clay to produce in a mixture with pure quartz

sand a very tenacious structure, able to resist the destructive effect not only of water, but of such alkali solutions as 0.05 *N* NaOH, appears remarkable. So is also the fact that the agregates prepared from pure quartz sand and Ca-clay possess the same "solubility" as aggregates prepared from Na-clay.

The recognition of the importance of Ca in the formation of soil structure has led to the denial of the possibility of the formation of tenacious structures (with the exception of tropical soils) outside of the ion adsorption of Ca cations by soil colloids. This conception is in contradiction to a number of facts which have thus remained without explanation. Thus, for instance, in the experiments of Tiulin on the effect of pressure on the formation of tenacious structures the saturation of kaolin with Na "alone, without applying pressure, increased, contrary to expectation, the number of water resistant aggregates in kaolin" (29, p. 47). According to the experiments of Vilensky, the tenacity of aggregates is increased in solonetz produced by treating chernozem with NaCl until a total displacement of Ca is reached (30). To the same class of phenomena, which cannot be adequately accounted for, belong also the origin of the so-called "passive slime" of Sokolovsky and the formation of the precipitate of "organo-mineral" colloids according to Tiulin, which is difficult or even quite impossible of separation.

The following remarkable observation has been reported by Nutting (13):

> An aggregate of fine and coarse particles together when moist takes on the properties of the finer particles alone. . . . The fine particles act simply as cementing material. . . . . Fine clay particles immersed in water in a thin layer between glass plates, under a microscope, may be seen gathering together in clumps. These clumps finally gather together in threads and ropes.

The author regards it as essential for the explanation of the cohesion between the particles gathered into clumps and threads to consider these as formations *possessing a fine structure, not unlike that of crystals, i.e., a structure, the separate elements of which are spatially oriented.* Indeed, observations of clay suspensions and of humate sols have shown that we are dealing here with spontaneous coagulation, resulting in the deposition of structures resembling mesomorphous formations (4, 5).

It is known that a suitable shape and size of the molecules are necessary conditions for the development of the mesomorphous state. Most investigators are of the opinion that only organic substances with rectilinear elongated molecules can be encountered in the mesomorphous state (31). Spontaneous anisotropy, determined by a definite disposition of molecules, is natural, however, also to inorganic bodies. Such is the case, e.g., of SiO_2 sols and of iron oxide hydrate reported by Quincke (20). A 3.2 per cent solution of SiO_2 purified by dialysis, after standing for 5 months, forms at the bottom of the container "about a hundred round lenses 2–3 mm. in diameter and possessing the characteristic structure of a spherulitic crystal" (20, p. 801). The SiO_2 solution having been decanted from the lenses deposited at the bottom "after a few more weeks produces new lenses" (20, p. 806). If it is subjected to

drying "the enriching in colloid solution of SiO_2, produces liquid deposits, first in the shape of thin lamellas perpendicular to the margin; later the same process is repeated at short intervals or periodically." As soon as these liquid lamellas solidify, "new lamellas are deposited normally on those formed earlier" (20, p. 808). These lamellas are made up of great numbers of "Shaumzellen" united one with another (20, p. 814), which are easily visible if a thick layer of SiO_2 is dried between a cover and an object slide. "The gel-like mass is divided into parts by fissures . . . parallel to the surface of fracture zones of large and small open Schaumzellen with confluent walls, normal to the surface of fracture . . ." (20, p. 820). Structural forms, closely resembling the aforementioned, are described by Quincke for iron oxide hydrate.

Particularly interesting is the work of Zocher (35) on the formation of structure in the sols of V_2O_5 and FeOOH, revealing a close resemblance to mesomorphous substances. Even earlier, the anisotropy of V_2O_5 sols was described by Diesselhorst and Freundlich (3), who designated such systems as anisotropic-amorphous ones. Szegvari (28) applied to the study of V_2O_5 sols the cardioid ultramicroscope with an azimut-diaphragm and found that at a certain concentration the sol particles are distributed in swarms of parallel oriented particles. The particles of such a sol are mobile and show Brownian movement. Zocher's (38) "tactosols" are formations of molecular groups arising in concentrated sols under the action of ageing, light, heat, and introduction of electrolytes; in the last case the ensuing coagulation is not a usual but rather an "arranged" one.

According to Ostwald's (15) opinion the concentrated solution of $BaSO_4$, the structural forms of which have been described by Weimarn (32), represents also a typical mesomorphous system. Weimarn finds a close resemblance between the $BaSO_4$ structures and those described by Quincke for the iron oxide hydrate, in spite of the chemical difference of the substances. Weimarn expresses the general opinion "that aggregate liquid crystalline condition is the general condition of matter."

THE STRUCTURE OF CLAYS

Vorländer's (31) view that inorganic substances are in most cases built up of spheric and not of linear molecules is not applicable to clays. For clays the tetrahedral sheets shape has become known, thanks to the investigations of Gruner (7, 8). Pauling (17, 18) established the similarity of cristallite forms of kaolinite, mica, and chloride. By analogy with substances with a leaf-like particle shape, for which the possibility of their existence in a mesomorphous state has been proved, one would think that clays also—when the shape of their crystallites is considered—may form a mesomorphous system. We know also that typical mesomorphous substances, such as stearine and palmitine acids, crystallize in the monoclinic system (34), i.e., in the same one as clay.

For the sake of obtaining a more definite idea of the matter in hand, it will be more correct to assume that clays by their nature are not mesomorphous sub-

stances, but that under certain conditions they can pass into a mesomorphous state[1] (15). Not less essential is the fact that this state of mesomorphous aggregation is acquired under the influence of water.

Sols of Na-clays isolated from various soil-forming rocks were used as the initial material for our study. Among these were loess-like loams, moraines, fluvio-glacial deposits, and Quaternary surface clays.[2] Two samples, taken from the shafts of the Moscow underground railway, represented clays from Jura and Carboniferous deposits.

The suspensions were prepared in the usual way from the rocks, ground into powder and screened through a 0.5-mm. sieve. Ca was displaced first by a saturated, then by a N NaCl solution. If the rock contained carbonates, as was usually the case, the latter were first dissolved in 2 per cent acetic acid. After the displacement of Ca, the rock was washed free of excess salt with distilled water as far as possible and then dialyzed in a parchment bag; after this, the fraction $<1\mu$ was elutriated. As the volume of the suspension was usually very large, we again coagulated the decanted suspension by a saturated NaCl solution, dialyzed the resulting precipitate of Na-clay, and thus obtained the final solution, free from salt and of approximately a 2.5–5 per cent concentration. It must be noted that in spite of a strict adherence to the rules of elutriation, we were unable to free these clay suspensions completely from very small quartz grains about 2–5μ in size, contaminating them, as could be seen under the microscope. These small quartz grains were not precipitated even when the time of elutriation was increased. To obtain clay suspensions entirely free from contamination by quartz grains we had to elutriate the suspension for a second time, diluting it greatly and increasing considerably the time of elutriation. Hence, suspensions prepared by the usual method of elutriation are always contaminated by coarse particles, this contamination being the greater, the higher the concentration of the suspension. The same thing was found by Hanley (10) in respect to pure quartz suspensions.

[1] According to Friedel, mesomorphous bodies are spontaneous and always anisotropic, whatever the conditions of their creation. Their anisotropy is manifested in double refraction, which may be very strong. Mesomorphous substances approach the amorphous ones in that they do not possess a fascicle of planes, having the properties of vectorial discontinuity, following the law of Haüy. The same substance may be encountered in two phases—the smectic and the nematic—the limits of the stability and the molecular structure of which form two intermediate points between the crystalline and the amorphous states. These phases are characterized by their absolute discontinuity.

The nematic phase (νῆμα—filament, thread) is a condition in which the molecular axes are arranged parallel to one another. The smectic phase (σμῆγμα—soap) is a condition in which the molecules are not only lying parallel to the axis but lie at equal distances in this direction. It is necessary to distinguish the mesomorphous state from mesomorphous substances; the former is not connected with the character of the chemical composition. Substances of most varying composition may assume the mesomorphous state under the influence of external conditions.

[2] The rock samples were obtained from the collection of Prof. Mirchink, to whom the author expresses his deepest gratitude for this kindness.

The X-ray investigation of all the clay samples was carried out by the powder method of Debye. Interference lines with uncertain borders were always obtained in the roentgenograms, indicating a considerable dispersity of the material, reaching at least 10^{-4} mm. The results of our measurements and their comparison with the data of other authors (7, 8, 11) show that the different minerals can be distinguished in the composition of clays: kaolinite was present in almost every sample, this mineral being probably their chief component. In several samples the chief mineral was found to be montmorillonite (bentonite) or galloisite. Besides these minerals, all the clay samples contained mica and quartz, the mica content being subject to great variations. In dilute sols clay particles can be seen at great magnification; they are about 10^{-3} mm. in size and show only slight Brownian movement. These particles are groups of still smaller ones, not larger than 10^{-4} mm., as may be conjectured from the interference lines of the roentgenograms. This tendency toward an aggregation becomes more marked with the "ageing" of clay sols. After a while, in some cases a very short time, a layer of a more concentrated sol, constituting a certain part of the total volume, is deposited at the bottom of the container. This precipitation is similar to that observed by Quincke (19) with the sols of many colloids ("the concentrated colloid solution"), and by Zocher (35) with V_2O_5 and FeOOH.

Sometimes the clay sol after prolonged standing coagulates spontaneously and an entirely clear liquid appears above a deposit, constituting about one-half of the total volume. Such a concentrated sol placed between a cover and an object slide shows a slight, diffuse double refraction, caused by the anisotropy of the colloid particles. Only those parts of the sol which are in contact with air-bubbles become optically oriented. The oriented area increases in size with time; its limits show a double refraction with an optical axis perpendicular to the longitudinal direction. Gradually the entire field of the sol is divided into areas differing in "density" from the more mobile and fluid medium surrounding them The limits of these "dense" areas become increasingly definite, and simultaneously the negative double refraction grows in the longitudinal direction. The delimitations of the structural formations are rectilinear, and their directions are almost perpendicular to one another and form a fine network within the thin layers of the gel. In the thick layers of the gel it is possible to obtain a laminated structure, which becomes clearly visible as a result of a great number of dark lines parallel to the longitudinal direction, visible also in natural light, as between the Nicol prisms. These lines show that the particles are distributed in separate layers lying at equal distances from one another, i.e., that we are dealing with the formation of the smectic phase (pl. 1, fig. 1). The structure resembles the "stries huileuses and batonnets" described by Friedel (4) and representing a modification of focal conic structures.

Such a laminated structure is typical of all of the clays tested—a total of 22 samples, all different in composition, age, and origin. All smectic bodies are,

according to Friedel, optically positive. The clays almost invariably show a negative double refraction, such as that found by Zocher and Jacobowitz (37) to exist in many smectic substances. In some cases clays show the positive phase, as for instance, when gels are subjected to freezing. It is possible that in these cases there is a gradual transition from the negative double refraction to the positive one, as was found by Zocher; this, however, could not yet be established for clays. The theory of this phenomenon is by no means clear.

The tendency of the molecules to occupy parallel positions corresponds to the condition of the least energy content. The parallel distribution in which every molecule lies along the direction of its axis represents the lowest order of the mesomorphous condition, the nematic structure. In clay sols water evaporation is the cause of the development of the smectic phase. The capillary force of the water film determines the association of separate groups of molecules into more complex formations. The excess of water ("swelling") results in the disappearance of the water film and of the smectic phase and in the appearance of the nematic phase. The boundary between these two phases is sharply marked, as has been established by Friedel. The transformation of one modification into the other is explained by the high dielectric constant of water, and this process is in complete analogy to heating, in which the fine-structural combination is also lost (25).

The nematic phase is observed in clays when they are in a greatly swollen state; this finding is similar to that of Rinne (24) in regard to the bromphenanthrene sulfonic acid. Figures 2 and 3, plate 1, show the nematic phase in a greatly swollen clay. Figure 2 shows the finely filamentous modification; figure 3, the granular one. As is well known, the nematic phase, according to Friedel (4) is represented by filamentous or by granular liquids ("liquides à fils ou à noyaux"). The nematic phase of clays shows strong double refraction with a negative optical sign.

Ornstein and Kast (14) advocate the swarm theory, according to which mesomorphous structures are not uniform over the whole macroscopic preparation. A uniform distribution exists only within small groups comprising about 100,000 molecules each. These small groups may be disposed irregularly in their chief directions, the interactions between the separate groups being weaker than those between the molecules within each such group. The arrangement of groups arises under the influence of external forces; hence, it is possible to speak of a polycrystalline structure. The independent groups may be considered as micro-crystals.

According to Stewart (27) the grouping of molecules takes place in every liquid; mesomorphous liquids are different only in that the size of their molecular groups, which Stewart calls "cybotactic groups," is greater. The arrangement of small groups into larger ones is not sufficient to cause the diffraction of X-rays (nematic bodies do not produce interference), but is sufficient to produce optical anisotropy.

It is noteworthy that Williams (33) long ago drew the attention of scientists

to the ability of clays to be precipitated spontaneously and observed the characteristic properties inherent to Zocher's "tactosols," i.e., the variability of the aggregate condition under the influence of light and heat.

THE STRUCTURE OF HUMATES

The shape of the particles of compounds of which the humates are composed is not known. Humates isolated from soil contain in most cases considerable amounts of clay and silica as contaminations. It is possible that humates are partly composed of compounds with elongated molecules, but the contaminations may change their state of aggregation.

The most suitable materials in respect to purity, i.e., minimal content of clay and silica, are humate samples isolated from peat (16), humates obtained in the decomposition of dung or hay,[3] or the products of the autolysis of the fungus *Aspergillus niger*.[4]

More concentrated sols were obtained by subjecting the humates prepared from peat, dung, and hay to evaporation in the incubator until a concentration of 6–8 per cent was reached.

In "ageing," humates do not show as high a capacity for spontaneous coagulation as do clays. Except for the products of autolysis of the fungus *Aspergillus niger*, which coagulate relatively soon, no deposit of a more concentrated sol is formed at the bottom of containers filled with approximately 0.2 per cent solutions of humates. From these signs it is evident that the tendency of humates toward spontaneous coagulation, even if it does exist, is insignificant.

Indeed, as observations show, a layer of concentrated solution of peat, dung, or hay humate between an object and a cover slide shows no double refraction either in parallel or in convergent light. Only where it borders air-bubbles does the fine limit-line show a weak double refraction with an optical axis normal to it. The same phenomenon is observed in much-swollen humate samples. When they dry, their capacity for aggregation is altered. If, for example, a drop of concentrated humate solution is dried on a slide, a picture typical of the mesomorphous condition is obtained, similar to the one described by Quincke (20, 21) for dried gels of SiO_2 and other colloids. Radial fissures, normal to the boundary, and separated zones of the drop, parallel to its border, are formed. The entire mass of the dried humate falls into separate parts; double refraction is shown only by the thin margins of the border line, their optical axis being normal to this line. The space occupied by the fissures is covered by a thin film of a weakly refracting material. Apparently we are dealing here with a similar double refraction (26), due to the depolarization of light, arising as a result of diffraction from very small microscopic or submicroscopic particles.

Peat humate, in drying between a cover and an object slide, is drawn toward

[3] The humates prepared from dung and hay were obtained from Z. J. Lein.

[4] The sample of this humate was obtained from F. J. Heltzer.

the borders into a layer of uneven thickness, due to a high surface tension. In the central areas of the preparation where the layer of the colloid is the thinnest, a network of fine filaments, similar to the one already described for clays, is formed. If the layer reaches a certain thickness, a structure of oily strips or rods is deposited, not different from that observed in clays (pl. 1, fig. 4). The double refraction is very weak, the borders of the delimiting lines of structural formation showing a greater refraction than the rest. The optical sign is positive, and the optical axis is always normal to the longitudinal direction. Similar structural forms are observed in humates prepared from dung and hay (pl. 1, fig. 5). At the rim of the cover slide, where the layer of the humate is considerably thicker, the structure in strips disappears and is substituted by forms arising from cracking (pl. 1, fig. 6), and differing from those previously described in that their delimiting boundaries acquire a rounded outline. Moreover, here we observe spherulites, producing no dark cross in crossed Nicols.

It is necessary to mention that peat and dung humates, photographed with X-rays, showed no indications of the presence of crystalline material in their composition. This circumstance cannot be regarded as the cause of the weak tendency of the humates toward mesomorphous aggregation. Cases are known in which substances amorphous in roentgenograms show double refraction in polarized light, e.g., filamentous alumina, according to the observations of Rinne (23).

Contamination by Ca salts causes humate sols to coagulate but in no way increases their aggregation as compared with Na-humates. Neither in shape nor in respect to double refraction does the smectic phase of Na-humates differ from the smectic phase of Ca-humates. Humate deposits obtained by precipitating humates with $Fe(OH)_3$ behave similarly. This observation conforms with the rule, discovered by Haber (9) and by Böhm and Niclassen (2), that, when a precipitate is formed quickly, amorphous formations arise which then are transformed gradually into crystalline ones. The humate obtained by autolysis of *Aspergillus niger* differs in its strong double refraction from the structures described. The characteristic feature of this humate is that in being dried between the cover and object slides it forms no homogeneous structure. The greater part of the preparation is occupied by a filamentous-like structure with a clearly visible distribution in layers and showing a strong negative double refraction (pl. 1, fig. 7). A strongly double-refracting fascicle of filaments is surrounded by a dark substance which cannot be rendered transparent by rotating the stage of the microscope. The appearance of amorphous substance around the smectic one may be explained by the lesser surface tension of the latter, the amorphous substance, as a result, being drawn to the borders. It is noteworthy that in convergent light this lamella seems to have a double-axis with hyperbolas passing out of the field of vision. Since the characters of the smectic phase are here distinctly pronounced and since it is difficult to suppose that we are dealing here with something else, it must be assumed, as an explanation of this anomalous structure, that the axes of the spa-

tial elements are perpendicular to the axes of the texture, as is characteristic in the case of twinning (37).

The dendrites in figure 8, plate 1, are a modification of the same stripes. Their appearance is due to the higher rate of crystallization in the presence of contaminations (1). The branches of the dendrites are surrounded by the same substance, which remains dark at rotation of the microscope stage. In a gypsum plate of the first order some parts of the dendrite structure are colored blue, while others are tinted yellow. When the stage of the microscope is rotated the former show a gradual transition from blue to yellow through the violet and red-brown tints, while the latter show the reversed course of color changes. Figure 9, plate 1, shows the spherulites, which do not produce a dark cross in convergent light and contain one or several grains of a double-refracting substance. They resemble those described by Quincke (21, p. 995). The whole area of the substance surrounding the spherulites possesses an unevenly distributed diffuse double refraction, the origin of which lies in the anisotropy of the colloidal particles of the humate.

TWINNING

Twinning phenomena are recognized by the increase and decrease of interferential coloring, periodical for different parts of the preparation (1). As observation shows, interferential coloring is regularly increased along the axis of the fascicle of filaments (pl. 1, fig. 1). While one such fascicle shows an increase in coloring, the adjacent one shows a decrease. When the stage of the microscope and the preparation with it is rotated a remarkably beautiful color change occurs simultaneously in the different sections of the preparation. These color changes show that we are dealing, not with different substances, but only with varying positions of one and the same substance.

It is interesting that twinning has been found, not only in the smectic phase of clays and humates, but also in the nematic phase during a highly swollen condition of clay (pl. 1, figs. 2 and 3).

Twinning phenomena in nematic liquids have been described by Friedel and Grandjean (6) and by Maugin (12). According to Zocher (36) the hematic phase can assume an enantiomorphous habitus, if it contains an optically active substance. It is possible that the quartz contamination, which cannot be eliminated from colloidal clay, may cause this twinning of structure. Another explanation is also possible, i.e., that clay belongs to the monoclinic system.

Bernauer (1) tested 480 substances and found that a considerable proportion of substances showing a capacity for twinning must be classed among the non-enantiomorphous ones, whereas a considerable percentage of substances showing twinning belong to the monoclinic symmetry.

Fine sand, mixed with water, gathers, when drying, into lines resembling in structure mesomorphous formations. The ranges of gathered sand are almost rectilinear, are sometimes curved, and cross almost rectangularly. The

quartz particles—right and left individuals—alternate, thus reminding one of Reusch's "mica systems." The rotation of the stage of the microscope with crossed Nicols produces no effect (pl. 1, fig. 10).

The phenomenon of anisotropy is complicated by that of twinning and is accompanied by a distortion of properties in various directions; this is probably the cause of the frequently observed deviations in the determination of the physico-mechanical properties of soils.

SUMMARY

The existence of aggregate phases in soil colloids alters essentially our conceptions regarding their behavior.

The electric properties of colloid particles, when they agglomerate into groups, are radically changed as a result of the shrinking of the ion envelope.

The properties determined by the nature of the absorbed cations are levelled out in the associated groups.

The possibility of spontaneous coagulation, explained by the prevalence of the forces of molecular attraction over those of electrostatic repulsion, is demonstrated.

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PLATE 1

Microphotographs of Clays, Humates, and Fine Sand

Fig. 1. Clay "Toida." Smectic phase. Polarized light. Magnification × 40.

Fig. 2. Clay "Toida" in highly swollen condition. Nematic phase. Polarized light. Magnification × 27.

Fig. 3. Cover clay "Makshany" in highly swollen condition. Nematic phase. Polarized light. Magnification × 27.

Fig. 4. Peat humate. Smectic phase. Natural light. Magnification × 40.

Fig. 5. Dung humate. Smectic phase. Natural light. Magnification × 40.

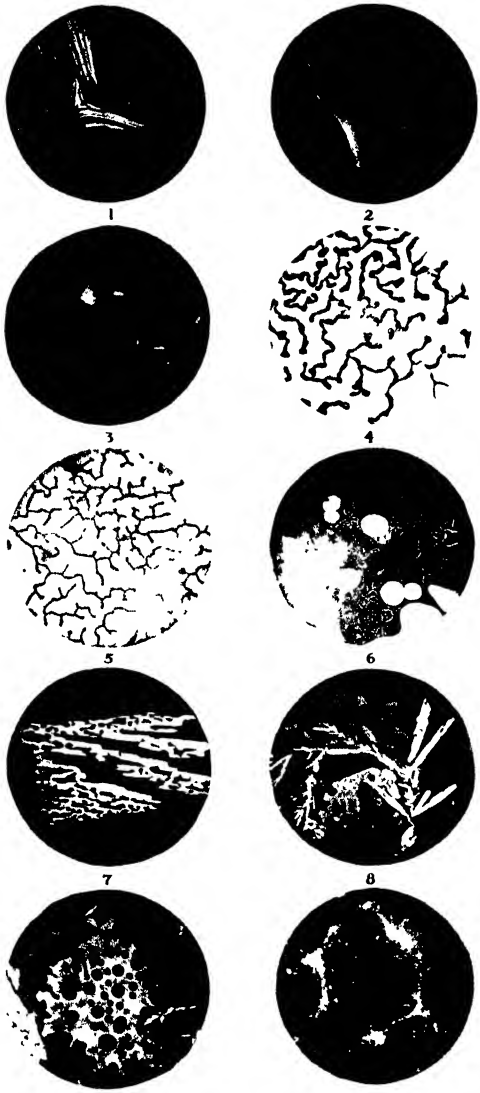
Fig. 6. Peat humate. "Figures of cracking" and spherulites. Natural light. Magnification × 40.

Fig. 7. Humate from autolysis products of *Aspergillus niger*. Smectic phase. Polarized light. Magnification × 40.

Fig. 8. Another position of the preparation shown in figure 7. Dendrite structure. Magnification × 40.

Fig. 9. Another position of the preparation shown in figure 7. Spherulites and diffuse double refraction. Magnification × 40.

Fig. 10. Rows of "gathered" sand. Polarized light. Magnification × 13.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BOOK REVIEWS

Theodor Brinkmann's Economics of the Farm Business. English Edition. Edited by MURRAY REED BENEDICT. University of California Press, Berkeley, Calif., 1935. Pp. x + 172.

Many books, monographs, and treatises have been added in recent years to the rapidly growing list of writings on agricultural economics. The present volume may have peculiar value in that it attempts to acquaint the American reader with the thinking of some of the leading agricultural economists in Europe. The editor in the preface says: "The writings of two men, Friedrich Aereboe and Theodor Brinkmann, have been especially representative of the best modern German thinking concerning the problems of agricultural economics, especially the problems of farm management. They represent an approach and point of view with which American students have not been widely familiar."

The book contains an editor's introduction; literature; three chapters, designated, respectively: General Consideration of the Types of Farming; The Levels of Intensity in Agricultural Production and The Orientation of Its Location; and Systems of Farming or The Orientation of the Locations of The Lines of Production; and an index.

It may also be noted from the editor's introduction that "One of the surprising features of Aereboe's and Brinkmann's treatment of these problems, as compared with the writings of many other German students, is the relatively small recognition of the effects of historical development on existing types of agriculture. This is, to be sure, recognized in the latter part of Brinkmann's treatment where he deals with dynamic aspects of the problem, but even there it is not fully developed. How much of the present form of agricultural production and of the present type of business unit are the result of the particular way in which the land of the United States was taken up or to the peculiar racial relationships which grew out of the era of slavery is an interesting phase of the background of farming types, and has so far had relatively little careful analysis."

Students of agricultural economics and the general reader will find much in this little book that is interesting and stimulating.

Feeds and Feeding. By F. B. MORRISON. The Morrison Publishing Company, Ithaca, New York, 1936. Pp. vi + 1050, illus. 204, tables 10.

This is the twentieth edition of a work which originally appeared in 1898. Its author was Dean W. A. Henry of the University of Wisconsin. Since few, if any, of our agricultural textbooks have enjoyed the popularity of this work,

it would be worth while to note here a portion of the brief historical record supplied in the author's preface to the twentieth edition. Dr. Morrison says:

The first edition of Feeds and Feeding, written by Dean W. A. Henry, and published in February, 1898, was received with immediate and widespread favor by practical stockmen and by professors and students of animal husbandry. In the United States and also in several other countries Feeds and Feeding was soon used more widely as a text book and as a reference book than any other book on livestock feeding.

During the years that have passed since 1898, nineteen editions of Feeds and Feeding have been issued, several printings have been made of each of the later editions. In 1910 the book was entirely rewritten by Dean Henry with the help of the author of the present edition. In 1915 and in 1923 complete revisions of the book were again brought forth. It was necessary for the present author to assume the responsibility for these revisions, since ill health had kept Dean Henry from active work for some time. He died in November, 1932, at the age of 82 years.

In the preparation of this entirely rewritten edition, the twentieth of the book, the author has spent much time during the past few years in compiling and analyzing the results of the many experiments on livestock feeding conducted in this and other countries. He has been aided greatly in this task by many investigators who have furnished him with information and with reports of experiments in advance of regular publication.

In order to present adequately the results, it has been necessary to make a considerable increase in the numbers of pages in the book. To save space and also to differentiate between the information of major importance and that of only local or minor interest, some parts of the text are set in a smaller type than that of the main portion. To aid students and others in their study of the book, review questions are now included at the end of each chapter.

It is the purpose of this volume to present in as simple and concise a manner as is possible the most important facts concerning the feeding, care, and management of the various classes of larger farm animals. Also, full information is given concerning the composition, use, and value of the many different feeding stuffs, especially those of importance in the United States.

The book is made up of three Parts entitled: Fundamentals of Animal Nutrition, Feeding Stuffs, and Feeding Farm Animals. The designations of the chapters are as follows: Part I: The Various Food Nutrients; The Digestion, Absorption, and Use of Food; Measuring the Usefulness of Feeds; Factors Affecting the Value of Feeds; Maintaining Farm Animals; Proteins—Minerals—Vitamins; Growth—Reproduction—Fattening; Production of Milk, Work, and Wool; Balanced Rations—Feeding Standards; and Economy in Feeding Livestock. Part II: Pasture and Hay; Silage and Soiling Crops; Legumes for Forage; Indian Corn and the Sorghums for Forage; The Hay and Pasture Grasses—the Cereals for Forage—Straw; Roots, Tubers, and Miscellaneous Forages; The Cereals and Their By-Products; Other Seeds and Their By-Products; Miscellaneous Concentrates; and Manurial Value of Feeding Stuffs. Part III: General Problems in Horse Husbandry; Feeds for Horses and Mules; Feeding and Caring for Horses; General Problems in Dairy Husbandry; Feeds for Dairy Cows; Feeding and Caring for Dairy Cows; Cost of Milk Production—Feeding Test Cows; Raising Dairy Cattle; General Problems in Beef Production; Feeds for Beef Cattle; Feed and Care of Beef Cattle—Methods and Cost of Beef Production—Veal Production; General Problems in Sheep Produc-

tion; Feeds for Sheep; Feeding and Caring for Sheep and Lambs—Fattening—Hot House and Spring Lambs—Goats; General Problems in Swine Husbandry; Feeds for Swine; and Feeding and Care of Swine. The appendix is made up of 10 tables entitled, respectively: Average Composition of American Feeding Stuffs, Digestible Nutrients, Mineral and Fertilizing Constituents, Digestion Coefficients; Estimated Net Energy Values of Feeding Stuffs; Morrison Feeding Standards for Farm Animals; Mineral Matter in Typical Feeding Stuffs and in Farm Animals; Vitamin Content of Feeding Stuffs; Weight of Concentrates and Other Feeds; Example Rations for Farm Animals; Factors and Constants for Valuing Feeds; Grain Feeding Tables for Dairy Cows; and Estimating Weights of Dairy Cattle from Heart Girths.

The author has placed his many readers under deep obligation to himself for having guided the evolution of one of our most valuable agricultural texts and reference books. He has dealt with an enormous mass of material and has known how to sift the wheat from the chaff. He has rendered a notable service to teachers, students, and farmers as well as to a not inconsiderable number of individuals not directly connected with our agricultural industry. Readers abroad, as well as those in the United States, will be ready to acknowledge their debt to him.

Biologie des Eisen—und Mangankreislaufs. By Paul Dorff. Verlagsgesellschaft für Ackerbau, Berlin, 1935. Pp. 116, illus. 32. Price 7.50 RM.

The student of microbiology, and especially of soil microbiology, will be greatly interested in the contents of this little volume. The cycle of iron and manganese in nature is of utilitarian as well as scientific interest. The origin and scope of the work are explained by the author, who notes:

> The work described in this book was carried out partly in the Biological Department of the Prussian State Institute for Water, Soil and Air Hygiene in Berlin-Dahlem and partly at the University of Lund in Sweden. Part I deals with the iron cycle. Both the regional occurrence of iron-depositing bacteria and the types of waters in which they occur in Småland and Northern Germany are described and compared; the text is well supplemented by illustrations. Samples of iron from the tropics and South-Eastern Europe are also discussed. It was found that numerous varieties of these bacteria are of very wide occurrence. In the micro-zones formed by these bacteria in the different layers of mud deposits, a large number of different varieties are contained; a table is given showing the rarer of these iron bacteria. The chemical processes involved in the bacteriological oxidation of iron carbonates are described; on the other hand a satisfactory solution of the problem of bacteriological oxidation of humates has not yet been found. In another section the technique used in research work on iron-depositing bacteria, especially the preparation of cultures (glass slides) is discussed in detail.
>
> The section on lake and swamp iron ores is well illustrated and contains a short description of the formation, chemical composition, distribution and exploitation of such ore deposits. Special mention is made of the districts in which the author made his studies. A geological map of the floor of a Swedish lake serves to demonstrate the conditions under which the iron deposits are formed. Of special interest to those concerned with the operation of water works and mineral springs is the description of the results of the activity of such iron and manganese depositing organisms as it contains many helpful hints on the solution of many hygienic and operation problems met with such concerns. Measures are suggested for the prevention of

many difficulties connected with agricultural drainage which often result from the activity of such organisms.

The author concludes with a discussion of the nonbiogenic formation of iron and manganese deposits, with special reference to pan formation in soils, to which many crop failures are due. The book is also provided with an exhaustive bibliography containing 305 references to the current scientific literature up to 1935, and should be of interest and use not only to hydro-biologists but also to geologists, agriculturists, and those concerned with the operation of water works.

Annual Review of Biochemistry. Volume V. Edited by JAMES MURRAY LUCK. Annual Review of Biochemistry, Ltd., Stanford University P. O., California, 1936. Pp. ix + 640. Price $5.00.

This is the fifth volume of the Annual Reviews of Biochemistry. It is noted in the preface that

> Almost every field of biochemistry continues to receive extensive inquiry and the number of papers calling for careful appraisal steadily increases. Selection of the papers from which a review is to be woven, judicious weighing of the evidence presented, and synthesis of the material into a readable survey is a most difficult task. Perhaps the most unhappy feature is the necessity of leaving unmentioned a considerable number of excellent papers which temporarily must be placed aside because of the exigencies of space or because they pertain to subjects beyond the limits of the survey.

The mass of scientific data reviewed in this volume is truly enormous. The nature of the contents may be best indicated by the topics listed and the name of the author of each section. These are given herewith: Oxidations and Reductions—D. E. Green and D. Keilin; Enzymes—J. H. Quastel; X-Ray Studies on the Structure of Compounds of Biochemical Interest—O. L. Sponsler and W. H. Dore; The Chemistry of the Carbohydrates and the Glucosides—W. N. Haworth and E. L. Hirst; The Chemistry of the Acylic Constituents of Natural Fats and Oils—T. P. Hilditch; The Chemistry of the Proteins and Amino Acids—C. Rimington; The Chemistry and Metabolism of Compounds of Sulfur—V. du Vigneaud and H. M. Dyer; Chemistry and Metabolism of Compounds of Phosphorus—R. Robinson; Carbohydrate Metabolism—I. L. Chaikorr; Fat Metabolism—E. F. Terroine; Metabolism of Amino Acids—H. A. Krebs; Mineral Metabolism—E. B. Hart and C. A. Elvehjem; Clinical Applications of Biochemistry—J. P. Peters, C. L. Robbins, and P. H. Lavietes; The Hormones—E. A. Doisy and D. W. MacCorquodale; The Water-Soluble Vitamins—H. von Euler; The Fat-Soluble Vitamins—E. V. McCollum; Nutrition—A. H. Smith; Liver and Bile—A. C. Ivy and L. A. Crandall, Jr.; Comparative Biochemistry of the Vertebrates and Invertebrates—F. Kutscher and D. Ackermann; Animal Pigments—J. Roche; Metabolism of Carbohydrates and Organic Acids in Plants—W. Ruhland and J. Wolf; The Biochemistry of the Nitrogenous Constituents of the Green Plants—G. T. Nightingale; The Rôle of Special Elements (Boron, Copper, Zinc, Manganese, etc.) in Plant

Nutrition—P. Moze; Bacterial Metabolism—A. J. Kluyver; Soil Microbiology—S. A. Waksman; and Biochemistry of Fungi—N. N. Iwanoff and E. S. Zwetkoff.

It is gratifying to note that a field of science undergoing such rapid expansion has been dealt with so adequately by the editorial committee and their collaborators. Workers in the field of biochemistry will use Volume V, as they have used the other volumes, with much satisfaction to themselves. No modern library can afford to be without a set of these reviews.

Biological Effects of Radiation. 2 Volumes. Edited by BENJAMIN M. DUGGAR. McGraw-Hill Book Company, Inc., New York and London, 1936. Pp. (vol. 1) x + 1–676, (vol. 2) vii + 677–1343. Price $12.00.

This two-volume work was made possible "by financial support from the Committee on Radiation through contributions received for the radiation program by the National Research Council from the General Education Board, the Rockefeller Foundation, and the Commonwealth Fund." It is noted elsewhere in the Preface that "The 'Biological Effects of Radiation' constitutes a collective contribution that has been developed as a by-product from one of the activities of a committee now known as the Committee on Radiation, in the Division of Biology and Agriculture, National Research Council."

The topics dealt with in the several chapters and the authors are as follows: Photons and Electrons, by Karl K. Darrow; Measurement of X-Rays and Radium, by Lauriston S. Taylor; Ionization and Its Bearing on the Biological Effects of Radiation, by G. Failla; Measurement and Application of Visible and Near-visible Radiation, by F. S. Brackett; The Intensity of Solar Radiation as Received at the Surface of the Earth and Its Variations with Latitude, Altitude, the Season of the Year and the Time of Day, by Herbert H. Kimball and Irving F. Hand; Statistical Treatment of Biological Problems in Irradiation, by Lowell J. Reed; Photochemistry, by Farrington Daniels; The Effect of Radiation on Proteins, by Janet Howell Clark; Radiation and the Vitamins, by Charles E. Bills; The Effects of Irradiation on Venoms, Toxins, Antibodies, and Related Substances, by S. C. Brooks; The Effects of Radium and X-Rays on Embryonic Development, by Elmer G. Butler; Effects of X-Rays and Radium upon Regeneration, by W. C. Curtis; The Biological Effectiveness of X-Ray Wave-Lengths, by Charles Packard; The Physiological Effects of Radiation upon Organ and Body Systems, by Stafford L. Warren; Short Electric Wave Radiation in Biology, by G. Murray McKinley; Biological Effects of Alpha Particles, by R. E. Zirkle; Motor Responses to Light in the Invertebrate Animals, by S. O. Mast; The Action of Radiation on Living Protoplasm, by L. V. Heilbrunn and Daniel Mazia; Photoperiodism, by W. W. Garner; Plant Growth in Continuous Illumination, by John M. Arthur; The Effects of Light Intensity upon Seed Plants, by Hardy L. Shirley; Effects of Different Regions of the Visible Spectrum upon Seed Plants, by H. W. Popp and F. Brown; Effect of the Visible Spectrum upon the Germination of Seeds and Fruits, by William

Crocker; The Effects of Visible and Ultra-violet Radiation on the Histology of Plant Tissues, by J. T. Buchholz; Some Infra-Red Effects on Green Plants, by John M. Arthur; The Effect of Ultra-violet Radiation upon Seed Plants, by H. W. Popp and F. Brown; The Effects of Radiation on Fungi, by Elizabeth C. Smith; The Problem of Mitogenetic Rays, by Alexander Hollaender; Effects of X-Rays upon Green Plants, by Edna L. Johnson; The Effects of Radium Rays on Plants, by C. Stuart Gager; The Light Factor in Photosynthesis, by H. A. Spoehr and J. H. C. Smith; The Influence of Radiation on Plant Respiration and Fermentation, by Charles J. Lyon; Growth Movements in Relation to Radiation, by Earl S. Johnson; Chlorophyll and Chlorophyll Development in Relation to Radiation, by O. L. Inman, Paul Rothemund and C. F. Kettering; Radiation and Anthocyanin Pigments, by John M. Arthur; Effects of Radiation on Bacteria, by B. M. Duggar; The Effects of Radiation on Enzymes, by Harold A. Schomer; Induced Chromosomal Aberrations in Animals, by Theodosius Dobzhansky; Radiation and the Study of Mutation in Animals, by Jack Schultz; Induced Mutations in Plants, by L. J. Stadler; Induced Chromosomal Alterations, by T. H. Goodspeed; Induced Chromosomal Alterations in Maize, by E. G. Anderson; and Biological Aspects of the Quantum Theory of Radiation, by John W. Gowen. The book contains also a subject index and an alphabetical list of contributors.

The editor and his collaborators have done their work faithfully and effectively. They have made a distinct and important contribution in a field of science which is attracting increasing interest. They have laid the foundation for further growth in the body of knowledge for which they have labored to the advantage of all students of the subject.

An Outline of Biometric Analysis. By Alan E. Treloar. Burgess Publishing Company, Minneapolis, Minnesota, 1936. Pp. 193. Price $3.10.

The specific nature of this treatise is indicated, in part in the author's preface. The following may be quoted:

> The present outline aims to provide in the simplest possible terms, the introductory reasoning that may lead to the establishment of that knowledge and conviction. It deals with principle rather than theoretical detail. The discussion of Part I is devoted to the simplest cases of univariate and bivariate frequency distribution, and purposefully avoids as far as possible the controversial topic of "small sample" analysis. Parts II and III are concerned with the subject matter of two more advanced courses dealing with correlational analysis and statistical estimation. These latter parts assume that an increased facility in algebraic reasoning, and a greater willingness to accept the results of calculus derivations, spring from the assimilation of the earlier discussions.

The contents are arranged in three parts. The individual chapters are designated, respectively: Part I—Introductory; Quantitative Data and Symbolic Representation; The Distribution of Frequency; The Measurement of Type; The Measurement of Dispersal; Moments and Frequency Curves; The Errors of Random Sampling; Probability and the Binomial Series; The X^2 Cri-

terion; The Coefficient of Correlation; Rectilinear Regression; The Error of Estimate; and Significant Correlation. Part II—Differences and Correlation; Partial Correlation; Multiple Rectilinear Prediction; The Correlation Ratio; Curvilinear Regression; The Coefficient of Contingency; Correlation from $(2xm)$ Fold Tables; Correlation Between Ranks; and Intra-Class Correlation. Part III—Introductory; The Distribution of Means; The Distribution of Differences Between Means; The Distributions of s^2 and of s; Fisher's z Distribution; "Student's" Z (or t) Distribution; The Joint Distribution of $\bar{x}$ and s; The Analysis of Variance; and The Distributions of Correlation Coefficients.

In addition there are an appendix containing four tables and a summary of formulae. This is followed by an adequate index.

The rapid increase in the importance of statistical data in the various fields of agricultural science will emphasize the helpfulness of this work. It offers distinct values to the teacher and student, as well as to the investigator, of biological phenomena.

Solon Robinson—Pioneer and Agriculturist. Volume I. 1825–1845. Edited by HERBERT A. KELLAR. Indiana Historical Bureau, Indianapolis, 1936. Pp. xxv + 582, illus. 10.

This publication represents Volume XXI of the Indiana Historical Collections. In his foreword to the book, Christopher B. Coleman notes:

> The inception of the publication of writings of Solon Robinson came about in 1926 at a meeting of the American Historical Association at Rochester, New York. In the session devoted to an "Agricultural Who's Who in the Ante-Bellum Period," the statement of Herbert A. Kellar that Robinson was the most important agricultural writer of that period in the North met with general acceptance. Robinson's extensive travels in the South, also, his observations upon the plantation system, and his efforts to work up common agricultural interests in both sections were of national significance. The late Ulrich B. Phillips, of Yale University, whose judgment upon such matters commanded the greatest respect, in expressing his agreement with Mr. Kellar, urged the publication of Robinson's writings. His interest in the project continued down to the time of his last illness. There have also been recurrent suggestions in the Agricultural History Society that such a publication would be a valuable contribution to the history of agriculture in the United States.

Reference may also be made to the preface by Herbert A. Kellar. He says:

> In the course of a long and active interest in the history of American Agriculture, I have been impressed with the personality and achievements of a group of men living for the most part in the ante-bellum period, whose whole-hearted and unselfish devotion to the cause of agricultural improvement won them national recognition in their own time. As a nation we shall be fortunate if in future we can point to the equals of such men as John Taylor of Caroline, John S. Skinner, Edmund Ruffin, Jesse Buel, Martin W. Philips, Thomas Affleck, Andrew Jackson Downing, John S. Wright, Benjamin P. Johnson, and last, but not least, Solon Robinson. Biographies of Taylor have been written by William E. Dodd and H. H. Simms, and Ruffin has his chronicler in Avery O. Craven. I take pleasure in presenting Solon Robinson of Indiana. You will find that he was a man of parts.

The book contains a list of documents dated within the period 1825 to 1845. These portray effectively the economic and social status of the United States, which at that time was still in its pioneering period. The reader will gain a distinct understanding of the environment under which the agriculture, commerce, and industry of that region evolved, the obstacles which had to be overcome, and the atmosphere under which generations of men and women grew into greater regional and national responsibility.

Sulfuric Acid Manufacture. By ANDREW M. FAIRLIE. American Chemical Society Series. Reinhold Publishing Corporation, New York, 1936. Pp. 669, figs. 187, tables 67.

Sulfur, sulfuric acid, and their derivatives are products of major concern to agriculture, industry, and commerce. They occupy an important place in the import and export business of many countries. For this reason, this book may be accepted as having large and peculiar value.

It is noted in the general introduction that "By arrangement with the Inter-allied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects." It is noted elsewhere that "The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations."

It may also be noted from the preface that "Foreign books on sulfuric acid manufacture have been pronounced deficient in the space alloted to the consideration of American practice. This book may be criticized abroad on the ground that American practice has been stressed too much. However, it is believed that in the contact processes American practice, during the period 1925 to 1935, has progressed farther than the foreign practice, and that there is justification for emphasizing the advances made in the United States."

The contents of the book are best indicated by the names of the chapters which follow: Historical; Chemistry and Theory Relating to Sulfuric Acid Manufacture; Construction Materials; Production Materials; Burners, Roasters and Furnaces; Burner- or Furnace-Gas; Nitration Processes—Methods of Nitration; Glover Tower; Lead Chambers and Chamber Substitutes; Recovery of Nitrogen Oxides; Auxiliary Equipment of a Nitration-Process Plant; Complete Plant Design—Operation of a Nitration-Process Plant; Purification and Concentration of Sulfuric Acid; Contact or Catalytic Processes. General Discussion; The Composition of Burner- or Furnace-Gas. Cleansing, Cooling and Drying the Gas; Blowers, Preheaters, Heat-Exchangers, Converters; Catalysts; Comparison of Platinum and Vanadium Catalysts; SO_3-Gas Coolers; SO_3 Absorbers, Oleum Manufacture, Miscellaneous Contact-Process Equipment; Complete Contact Plant. Operation. Methods of Control of Process; Special Types of Contact Plant, Individual Installations; Mixing and

Shipping Sulfuric Acid, Hazards and Safety Measures, Costs and Cost-Accounting, To Buy or To Build, Choice of Process, Trends in the Industry. The appendixes contain a wealth of timely and useful information, and the index is an adequate one.

Dictionary of Terms-Relating to Agriculture, Horticulture, Forestry, Cattle Breeding, Dairy Industry, and Apiculture in English, French, German, and Dutch. Compiled by T. J. BEZEMER. The Williams & Wilkins Company, Baltimore, 1935. Pp. vii + 294.

The terminology in the fields named in the title is growing rapidly. New terms are being introduced to a point where the student of agriculture and of the biological sciences is very much in need of new information and of clear definitions. As is noted in the publishers' preface:

> Students of Agriculture, Horticulture, Forestry, Cattle Breeding and those engaged in the Dairy Industry are repeatedly obliged to consult textbooks or works of reference in a foreign language. In searching for a translation of the technical terms used in their special subject of study they find that the standard dictionaries, if they give the word at all, fail to provide a satisfactory equivalent. This applies not only to technical terms, but also to common words relating to Agriculture, Horticulture, Forestry, Cattle Breeding and the Dairy Industry which are not used in their common or general sense, but with a more restricted or specialized meaning.

In the foreword by the editor, it is noted:

> As appears from the Publishers' Preface, this work is the first dictionary of its kind in this field, and it is as such it should be judged by the users The great number of auxiliary sciences which have to be studied for the practice of agricultural economy rendered it imperative to make a selection of the words to be included in the work.

This dictionary is to be commended to a wide circle of students. It will be found useful by teachers in our universities, colleges, and secondary schools; by thousands of workers in the field of agricultural extension; and by the great agricultural constituency who are engaged in the production of crops and the raising of farm animals.

JACOB G. LIPMAN.

A NEW METHOD FOR DETERMINING THE POROSITY OF THE SOIL

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Agriculture College of Sweden, Uppsala

Received for publication August 18, 1936

In the methods for determining the porosity of the soil worked out by Kopecky (3), Burger (2), et al., a fixed volume of soil is divided into soil substance, soil water, and soil air. The volume of the whole sample is equal to that of the cylinder used for taking the sample; the water content is found by drying the soil; and the volume of the soil substance is determined by multiplying the soil weight by the specific gravity of the soil. The air content in the soil sample will then be found by difference:

$$100 - (\text{per cent soil substance (vol.)} + \text{per cent soil water (vol.)}) = \text{per cent air (vol.) in the soil.}$$

Other principles have also been worked out, for example that of Nitzsch (16), who calculated the volume of the soil substance as the difference between the weights of the soil in air and under water, or that of Dojarenko (4), who determined the water content by shaking the soil in alcohol and measuring the soil volume directly.

These methods are laborious and, as the air content in all of them will be found by difference only, the value will be more or less doubtful when dealing with soil cultivation investigations, in which the air content is the prime factor.

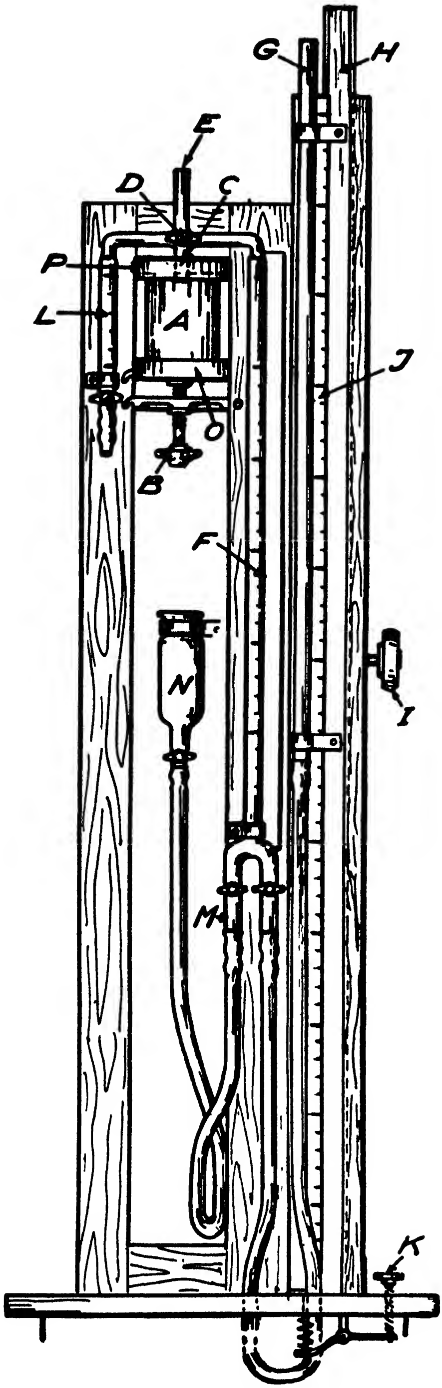
Recently we described a new method (8) by which the air porosity of the soil can be measured directly. Soon afterward, Nitzsch (7) also brought out a similar method.

In our method, rustless steel cylinders are used for taking the soil sample in the same way as that used by Burger (2), Blohm (1), Nitzsch (6) et al., but for determining the air porosity a special apparatus, "the porosimeter," is used.

DESCRIPTION OF APPARATUS

The soil samples are taken, in their natural condition, with the aforementioned cylinders and put into cylinder *A* (fig. 1). The cylinder is standing on a plate *O* which can be moved by a screw *B* and fixed airtight to a rubber packing *P*. Through this packing a glass tube *C* passes; by means of the tap *D* the cylinder *A* can, as desired, be connected with the atmosphere *E* or with the tube system *F-G*, which is filled with mercury.

The glass tube *F*, mounted on a mirror, has a volume of 50 cc. and is divided



A Cylinder in which the soil samples are measured.
B Screw for fixing plate *O* with cylinder *A* to rubber packing *P*.
C Glass tube.
D 3-way-tap.
E Glass tube to the atmosphere.
F Glass tube divided into 0.1-cc. units.
G Glass tube on a movable sliding piece *H* connected by a rubber tube with the glass tube *F*.
I Screw to move *H*.
J Reflecting glass scale.
K Screw for adjusting *J*.
L Correction tube.
M Rubber tube to adjustment vessel *N*.

Fig. 1. Diagram of the Porosimeter

into 0.1-cc. units. A rubber vacuum tube connects it with the glass tube G, which is mounted on a sliding piece H. By a screw I this can be moved upward and downward. Behind G there is a scale marked on a mirror, which can be adjusted by screw K. The total volume of the system L-A-F can be corrected by means of glass tube L to whole cubic centimeters for easier calculation. The amount of mercury in the system F-G may be adjusted from the vessel N and the rubber tube M.

If we connect the tube F, through tap D, with the atmosphere, the mercury will have the same level in both tubes F and G. If, on the contrary, the tap is set to connect tube F with the cylinder A, and we move tube G upwards with screw I, we will have an excess pressure in A and tube F. This excess pressure can be read off on the scale J.

METHOD OF DETERMINATION

The following terms are used:

- v_a total volume of cylinder A and glass tube F to the 0-mark.
- v_j total volume of the soil (in wet samples = soil + water).
- b barometric pressure.
- t pressure difference.
- f volume variation.

When using the porosimeter, we first determine v_a. For this purpose it is necessary to begin with a relatively low reading of the mercury in tube F, and to reduce the air volume f cc. by moving the tube G upwards. The volume will then be $v_a + f$ at the beginning of the measurement, and the pressure b cm. of mercury. When the mercury in the tube F is at the O-mark, the air volume is v_a and the pressure $b + t$ cm. According to Boyle's law we will have the following equation:

$$(v_a + f)\cdot b = v_a\,(b + t)$$

then

$$v_a = \frac{bf}{t} \tag{A}$$

If we put a soil sample in the cylinder A the volume v_j will be found in an analogous way

$$(v_a - v_j + f)\cdot b = (v_a - v_j)\cdot(b + t)$$

and then

$$v_j = v_a - \frac{bf}{t} \tag{B}$$

By determinations with the porosimeter, therefore, the air volume is measured and the soil volume calculated as we have already shown.

The apparatus can also be used for determining the specific gravity of the soil; if v_i in formula (B) refers to dry soil and its weight is a gm. the specific gravity will be

$$s = \frac{a}{v_i}$$

If, on the contrary, the specific gravity of the soil is known, the porosimeter can be used for determining the water content of the soil. Assume a soil sample of a gm., with a specific gravity s, volume v_i cc., and p per cent water content by weight, then the volume of the water + soil substance is equal to the total volume of the wet sample according to the following equation:

$$\frac{p}{100} \cdot \frac{a}{1} + \frac{(100 - p)}{100} \cdot \frac{a}{s} = v_i$$

then

$$p = \frac{100\,(v_i s - a)}{a(s - 1)} \text{ per cent (weight)} \qquad (C)$$

PRACTICAL APPLICATION

In our earlier paper (8) we discussed the principle of the apparatus and showed its applicability. In the present paper actual figures are given for comparison of this method with existing methods. These investigations have been done on different soils, varying from strongly marked sandy and peaty to heavy clay soils.

Experiments were first conducted on a sandy soil, poor in humus, of the following texture:

	per cent
Coarse sand (2–0.2 mm.)	68 3
Fine sand + coarse silt (0.2–0.02 mm.)	19 4
Fine silt (0.02–0.002 mm.)	1 8
Clay (<0.002 mm.)	7 6
Organic matter (loss on ignition with reduction according to the clay content)	2.9

Of this soil six samples were taken from the surface layer in their natural condition, to a depth of 10 cm., with our rustless steel cylinders. The samples were first weighed, and their porosity, i.e., air space, was determined in the porosimeter.

For comparison with existing methods, the samples were then dried at 110°C., to constant weight, and weighed. The dry soil was once more tested in the porosimeter, and the specific gravity of the soil was determined according to the method of Zunker (5). The figures obtained by weighing the soil before and after drying enabled us to calculate, in the usual way, that part of the pore space which is filled with air. In table 1 the values calculated in this way are compared with those obtained in the porosimeter. In the second column the total volumes of the soil samples are tabulated, in order that the reader may

use the figures for other calculations. The absolute agreement between columns 3 and 4 will depend a little on the value of the specific gravity of the soil, which is used for calculating the figures in column 3. It can be seen that the values in column 3 are, generally, lower than those in column 4. This difference, in itself of no importance, is due to the fact that the value for specific gravity obtained in the pyknometer was a little too low, because some plant residues were floating. This is confirmed by the fact that another determination of the specific gravity on the dry soil in the porosimeter gave an average value of 2.69 from six determinations, whereas that obtained in the pyknometer

TABLE 1

Comparison of the values obtained on sandy soil in the porosimeter with those calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	PORE SPACE CALCULATED IN THE USUAL WAY	PORE SPACE FOUND IN THE POROSIMETER
	cc.	*cc.*	*cc.*
25	665	256	257
26	673	230	236
27	673	217	224
28	670	244	252
29	676	249	256
30	679	267	273

TABLE 2

Comparison of the water content of sandy soil calculated according to formula (C) *with that found by drying the sample at 110°C.*

NUMBER OF THE SAMPLE	WATER CONTENT ACCORDING TO (*C*)	WATER CONTENT DRIED AT 110°C.
	per cent	*per cent*
25	9 5	9.5
26	9 0	9.5
27	9.3	9.9
28	8.5	9 4
29	8.1	8.8
30	8 4	8.8

was 2.67. The figures in column 3 are calculated on the basis of the pyknometer value 2.67.

As an example of the application of formula (*C*), table 2 is given. If the volume of soil substance + water in the porosimeter has been found to be 407 cc. and the weight of the soil in the cylinder 943 gm., the water content in the soil will be determined as follows

$$p = \frac{100\cdot(407\cdot 2.69 - 943)}{943\cdot 1.69}$$

which gives $p = 9.5$ per cent.

In this case we have used the specific gravity obtained in the porosimeter, because the intention is to use only the porosimeter.

The agreement between the values in columns 2 and 3 is, perhaps, not so good as desired, in view of their small size; but the variation in column 2 is nevertheless only slightly greater than that in column 3.

The next experiments were conducted on samples from the surface, also to a depth of 10 cm., of a much finer sandy soil, of the following texture:

	per cent
Coarse sand (2–0.2 mm.)	6 8
Fine sand + coarse silt (0.2–0.02 mm.)	75 1
Fine silt (0.02–0.002 mm.)	4 3
Clay (<0.002 mm.)	9 1
Organic substance (loss on ignition with reduction)	4 7

The determinations were the same as those described for the sandy soil. The difficulty in obtaining one value for the specific gravity, representing the

TABLE 3

Comparison of the air space, of fine sandy soil, obtained in the porosimeter with that calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED, S. G. 2.63		AIR SPACE CALCULATED, S. G. 2.73		AIR SPACE FOUND IN THE POROSIMETER	
	cc.	*cc.*	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*
31	683	178	26 1	190	27.8	186	27 2
32	684	196	28 7	207	30 3	208	30 4
33	679	206	30 3	217	32 0	208	30 6
34	682	166	24 3	177	26 0	181	26 5
35	679	174	25 6	186	27 4	192	28 3
36	679	163	24 0	174	25 6	178	26 2
Average			26.5		28 2		28 2

whole sample, was, in this case, even greater, because of an increase in the amount of plant residues in the upper part of the sample. To show the possible differences, we give three values for the air space in table 3, one of which has been calculated on the basis of specific gravity 2.63, obtained by the pyknometer method; a second, based on the value 2.73, found in the porosimeter; and a third, from direct measurement in the porosimeter. The value 2.73 is also found by solving s in the formula (C), after inserting the value for water content found by drying the soil at 110°C.

For a comparison of our method with existing methods, columns 3–4 should be compared with 7–8 (table 3). Taking into account what has already been said regarding the difficulty of obtaining a representative value for the specific gravity of the whole sample, we have found it convenient also to make the corresponding calculation in columns 5–6, using the considerably higher value found in the porosimeter. The figures in columns 5–6 and 7–8 show the best

TABLE 4

Comparison of the air space, of heavy clay, found in the porosimeter with that calculated in the usual way

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED IN THE USUAL WAY	AIR SPACE FOUND IN THE POROSIMETER
	cc.	*cc.*	*cc.*
1	667	174	178
2	669	142	140
3	673	125	125
4	672	149	150
5	670	145	157
6	676	164	165
7	667	133	135
9	674	137	135
10	666	161	161
11	672	121	120
12	672	128	129
13	680	161	161
14	669	126	125
15	676	133	133
16	680	155	155
17	680	161	163
18	679	174	174

TABLE 5

Comparison of water content of heavy clay calculated according to (C) *with that found by drying*

NUMBER OF THE SAMPLE	WATER CONTENT, ACCORDING TO (C)	WATER CONTENT, DRIED AT 110°C.
	per cent	*per cent*
1	22 1	22 8
2	23.9	23.6
3	23 6	23.6
4	22 8	22.9
5	20 8	22 6
6	23 3	23 4
7	22 1	22 5
8	22 6	Spoiled
9	23 0	22.7
10	22 0	22 0
11	22 4	22.3
12	22.0	22 2
13	22.7	22 7
14	23.4	23.2
15	23 3	23.3
16	22.9	22.9
17	22.0	22.4
18	22.9	22.9

agreement and, in this case, even the same average percentage of air-filled pore space. When we are dealing with such a varying quantity as air space, however, the deviation between the different reiterations is necessarily large.

TABLE 6

Comparison of calculated air space in three different peaty soils with that found in the porosimeter

SOIL	NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	AIR SPACE CALCULATED	AIR SPACE, FOUND IN THE POROSIMETER
		cc.	*cc.*	*cc.*
Well-decomposed peat, s. g. 1.81	1	667	166	162
	2	669	148	145
	3	673	164	161
	4	672	164	162
	5	670	175	172
	6	676	184	183
Moderately decomposed peat, s. g. 1.73	7	667	97	97
	8	678	102	103
	9	674	115	113
	10	666	122	119
	11	672	157	156
	12	672	162	159
Slightly decomposed peat, s. g. 1.58	13	680	165	164
	14	669	174	173
	15	676	142	141
	16	680	79	74
	17	680	101	101
	18	679	131	127

TABLE 7

Comparison of the water content of a peat soil calculated according to formula (C) *with that found by drying the sample in a vacuum at 50°C.*

NUMBER OF THE SAMPLE	WATER CONTENT, ACCORDING TO (C)	WATER CONTENT, DRIED AT 50°C. IN VACUUM
	per cent	*per cent*
7	72.7	72.5
8	72.6	72.8
9	75 6	75.0
10	75 5	74.3
11	78 3	77.8
12	79.7	78.1

When this is considered, the difference between columns 3–4 and 7–8 will be within the limits of error.

Similar investigations were made on a heavy clay soil, the texture of which was as follows:

	per cent
Coarse sand (2–0.2 mm.)	5.3
Fine sand + coarse silt (0.2–0.02 mm.)	12.3
Fine silt (0.02–0.002 mm.)	18.4
Clay (<0.002 mm.)	51.5
Organic substance (loss on ignition with reduction)	10.1
Lime	2.4

The samples used were taken preliminary to a cultivation trial, where the porosity will be frequently checked with the porosimeter. The specific gravity was determined in the pyknometer and is, for an average of 12 determinations, 2.706 ± 0.008, on which value the figures in table 4 are based.

TABLE 8

Comparison of v_t values determined by increased pressure with those obtained by decreased pressure

INCREASED PRESSURE AFTER ATMOSPHERIC PRESSURE	DECREASED PRESSURE AFTER INCREASED PRESSURE	DECREASED PRESSURE AFTER DECREASED PRESSURE	INCREASED PRESSURE AFTER DECREASED PRESSURE
100	104 8	104 8	100 0
100	99 1	99 1	99 1
100	101 9	101 0	98.1
100	105 1	104 1	100.0
100	103.1	104 2	100.0
100	102 9	102 9	99 6
100	103 6	101 8	99.1
100	103 6	103.6	100.0
100	106 3	106.3	99.0
100	103 1	103 1	100 0
100	107.6	107.6	98.7
100	106 6	106 6	98 7
100	110 9	110 9	100 0
100	106 0	107 5	97 0
100	107 5	101 5	100 0
100	102 4	103 5	98.8
100	105 5	104 1	100.0
100	107 9	106 6	98 7

If sample 5 is disregarded, the calculated and the directly measured porosities are in almost absolute agreement.

In table 5 the water content, obtained by using the specific gravity figure of 2.706 ± 0.008 in formula (*C*), is compared with that found by drying the sample at 110°C. The agreement between the figures leaves nothing to be desired.

It is tiresome to determine the specific gravity necessary for calculating the figures in column 2, and we find it more convenient to dry a number of samples, after determination in the Porosimeter, and use the value for water content so found in formula (*C*), by which *s* may be solved. For hygroscopic soils, this procedure is undoubtedly preferable to working with quite dry soils in the

porosimeter. This was done on the 17 soil samples in table 5, and the specific gravity obtained was 2.716 ± 0.007. The difference between this value and that found in the pyknometer is thus 0.010 ± 0.011, i.e., no difference.

In order to prove the applicability of the porosimeter to organic soils, similar investigations were made on three unequally decomposed peaty soils. The calculated values in table 6 are based upon the specific gravity found in the pyknometer. The agreement between the two methods is remarkably good.

At present, we cannot adequately discuss the "degree of dryness" most suitable as a basis for determining the specific gravity of peaty soils. The

TABLE 9

Determination of air porosity in a heavy clay, under bare fallow, cultivated in two different ways

NUMBER OF THE SAMPLE	TOTAL VOLUME OF THE SAMPLE	TOTAL AIR SPACE	AIR SPACE IN PER CENT OF WET SOIL
	cc.	*cc.*	
Fallow I—soil compact			
1	688	131	19.0
2	699	76	10 0
3	688	62	9.0
4	689	62	9.0
5	696	48	6 9
6	684	91	13.3
			Av. 11.4 ± 4.3
Fallow II—soil loose			
7	684	243	35.5
8	688	199	28 9
9	695	233	33 5
10	692	198	28 6
11	688	168	24 4
12	690	171	24.8
			Av. 29.3 ± 4 5

values here used, however, refer to peat substances dried to constant weight in a vacuum (10 mm. Hg) at 50°C.

To complete the figures for peat, the water content calculated by formula (*C*) and that found by drying one of the peat soils have been brought together in table 7. The specific gravity used was determined in the pyknometer.

When using the porosimeter it is possible to work with either an increased pressure or a decreased pressure.[1] To ascertain whether we will obtain the

[1] In this case, the air porosity is calculated in the following way:

$$v_a = \frac{f(b-t)}{t}$$

same result in both cases we have determined v_i partly by increased pressure and partly by decreased pressure, as well as after increased and decreased pressure. The results of these investigations are made clear in table 8. The value of v_i which was obtained after atmospheric pressure was set at 100. The change of the air volume in all cases was 45 cc.

At a glance, the differences between the figures obtained by working with increased pressure and those found by using decreased pressure, appear to be great. This is mainly explained by the different effects of the increased and decreased pressure on the rubber packing. For particular investigations, where more than relative precision is required, the average value from one determination by increased and one by decreased pressure may be used to advantage.

As an example of the practical value of the porosimeter, some figures from a field investigation are given in table 9. The soil was a heavy clay, under bare fallow, cultivated in two different ways. Under one, the soil was closely compact, and under the other, relatively loose. After heavy rain, the water did not penetrate into the soil receiving the first type of cultivation but penetrated freely into the second.

The samples were taken at not less than 10-m. intervals and to a depth of 10 cm. The difference between the samples, therefore, is relatively large, but the differences between the figures from fallows II and I are 17.9 ± 2.8, leaving no doubt as to the structural differences after the two types of cultivation.

SUMMARY

This paper describes a new method for the direct measurement of air space in soils based on Boyle's law. The apparatus, the porosimeter, also permits calculation, in a simple way, of the specific gravity, water content, and soil substance.

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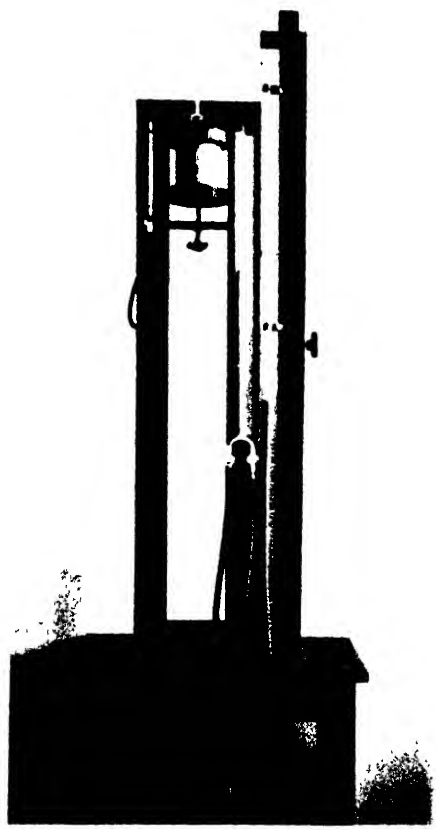
PLATE 1

The Porosimeter



AVAILABLE CALCIUM A FACTOR IN SALT BALANCE FOR VEGETABLE CROPS[1]

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The availability of calcium in a soil has a tremendous effect on the quality of growth of vegetable crops. The type of fertilizer materials which are used for the growth of vegetable crops and the manner in which the soil should be handled depend on the potential calcium supply. Vegetable crops differ widely, and even varieties differ in their nutrient requirement with respect to available calcium. Fertilizer residues have a marked effect on exchangeable and available calcium as well as on the reaction of light cultivated soils. Observations made on vegetable farms in New Jersey, located on the sandy soils, show that pH is not always a reliable indication of the available calcium and that many of these sandy soils which have pH values[3] of 6.0 to 6.6, where large applications of soda and potash have been made, may be very deficient in calcium and magnesium. For acid soils or soils that have not received heavy applications of inorganic fertilizers the reaction test is a fairly reliable index of lime requirement. Cultivated soils on which liberal quantities of chemical fertilizers have been used, however, require a specific calcium test which should be interpreted on the basis of soil type and exchange capacity to indicate reliably the need for calcium.

Crops grown on soils having a high pH but a low available calcium supply respond differently to available soil moisture from those grown on soils having a more favorable nutrient balance. Observations on crops during several years of dry weather showed that the degree of injury varied with different farms even though soils were very similar in type. Truck crops on dairy farms where manure was used showed much less injury during dry weather than did those on farms where certain chemical fertilizers were used. Of the growers using all inorganic fertilizers some fared better than others. Those growers

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of vegetable gardening.

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[3] All pH values quoted in this paper were determined either by the hydrogen electrode or by the Hellige Klett colorimeter.

who were following a fertilizer program of lime and acid-forming fertilizers were growing better crops during dry weather than were those who used acid fertilizers without lime or those who used a neutral fertilizer in which neutrality was brought about by other means than lime. It was common to find a luxuriant growth of vegetable crops on some farms as long as moisture was fairly well distributed, but to find those same crops very badly damaged during short periods of dry weather. In many cases there were differences in color of foliage and type of growth which could not be associated with any nutrient deficiencies. Similar symptoms were noticed on greenhouse tomatoes and cucumbers where an attempt was made to control the moisture supply. Greenhouse growers have to contend with a wide range of weather conditions because they grow crops the year around. The type of growth of plants grown in the greenhouse can be regulated to a great extent by controlling the water supply. Crops growing on dry soil very quickly indicate whether the soil solution is in good condition for optimum growth because, if the nutrients are not properly balanced, deficiency symptoms for certain nutrients may be encountered even though there is an abundant potential supply of a particular nutrient present. In view of the fact that available calcium has played such a large part in correcting greenhouse soil problems a number of preliminary experiments were conducted to study the relation of available calcium to type of growth principally of cucumbers, tomatoes, and lima beans, and a few other crops such as onions, celery, and carrots.

LOW CALCIUM SOILS AND THE RESPONSE OF PLANTS TO THE ADDITION OF CALCIUM SALTS

Soils from farms on which problems existed, but where the reaction of the soil seemed satisfactory, were brought to the greenhouse, placed in pots, and treated with various chemicals. Seeds or plants of certain crops were then planted in them. In practically all such cases, the soils were brought to the greenhouse because crops did not respond to side-dressings of ammonium sulfate or sodium nitrate; or if there was a response, the type of growth was unsatisfactory and the harvested crop was of poor quality. Poor germination of lima beans; crooked, poorly filled string beans; brown roots on most crops; rough rooted carrots; burned heart leaves of celery; light green spots on celery leaves; onions that did not keep well when harvested; and tomato fruits that did not ship well because they were too soft, were common symptoms. Usually when the marketable crop was of poor quality, the foliage also had certain peculiar symptoms which were not particularly characteristic of those caused by extreme deficiencies.

These soils were tested by means of the quick soil tests, as recommended by Hester (2), and were supplemented in some cases with microchemical tests on the plant tissue. These tests showed a low amount of available calcium (less than 100 p.p.m.—a few cases indicated a negative test) but the usual moderate amounts of all of the fertilizer nutrients present in a mixed fertilizer.

Very often high concentrations of potassium were found, or if potassium was not high, it was assumed from the growers' practice and a high pH reading that sodium probably was present in liberal amounts even though the concentration was not determined.

The technique in these soil pot cultures was to supply sodium nitrate, calcium nitrate, calcium chloride, calcium carbonate (limestone), superphosphate, gypsum, and in some cases potassium nitrate, dolomite, and calcium-magnesium silicate slag to separate pots as a preliminary trial. Growth of the plants was then observed. Usually the results of the preliminary trial were sufficiently convincing to warrant the making of recommendations. Where the problem involved a calcium deficiency, the more soluble calcium salts gave a better response than those having a lower solubility. Calcium nitrate was highly effective. If the pots were kept well watered, superphosphate proved very beneficial, and gypsum was satisfactory. If the soil was kept quite dry, calcium nitrate gave a very good correction, but calcium sulfate was ineffective. Superphosphate was effective but not so effective as calcium nitrate. One difficulty with calcium nitrate was the necessity of supplying it at 3-week to monthly intervals to obtain best results. Dolomitic limestone usually gave very good results, and the correction was usually better than with calcium carbonate.

On these calcium-deficient soils sodium and potassium nitrate had depressing effects on growth, the degree of injury (unless some form of calcium was supplied with it) depended on whether the soil had abundant potassium or whether in the past it had been supplied with comparatively large amounts of sodium nitrate.

LOW CALCIUM-HIGH POTASSIUM SOILS AND PLANT GROWTH

One of the experiments on plant growth involving a greenhouse soil containing low calcium and high potassium is given as an example.

Cucumbers when freely watered in the greenhouse made a rapid growth in certain sections of the beds but were not normal. The internodes were long, probably as a result of the large amounts of water needed to grow the crop. The foliage was light green, and the leaves were small. They were grown on strings up to wires 7 feet above the soil. When the growing tip reached the wire, many of the veins of the older leaves showed a brown, water-soaked condition, which was soon followed by dying and gradual drying of the whole leaf, with the result that the plants soon had only the tip leaves left. Mosaic-like symptoms were present and were associated with malformed growing tips and leaves. After it was decided that mosaic was not the cause[4] the plants were examined microchemically. The tissue in question was packed with potassium and nitrate, but calcium was entirely lacking. The roots were kinky and rough, and the cortex had sloughed off prematurely.

[4] Dr. S. P. Doolittle of the U. S. Department of Agriculture examined these plants and said there was no mosaic present, but that the disturbance resembled a nutritional one.

Tomatoes growing in another house were tested, and similar observations were made. The tomatoes were growing much better and were fruiting but were not making a desirable growth; it was apparent that they were being affected by a similar nutritional disorder. The foliage was dotted with light green spots.

A quick chemical test (2) showed a pH range of 5.8 to 6.6.[5] Less than 50 p.p.m. of calcium was present. Potassium was exceptionally high and fluctuated with the water supply. As soon as a test could be made after a heavy application of water, the potassium was found to be very low but just before water was again applied, when the soil was quite dry, the potassium concentration was found to be very high. Apparently very little of the potassium was in the colloid and was easily leached to lower levels. The phosphorus concentration was approximately 70 p.p.m. Only a trace of magnesium was found, but the nitrate concentration was high. The soil baked very hard when dry and was very slippery when wet. It was classed as a medium sandy loam of a grayish black appearance although organic matter was comparatively low. A hardpan from one-half to 2 inches thick had formed under the plow depth, although drainage was satisfactory in most cases. This soil had been in use for 16 years.

The result of these observations indicated that a deficiency of calcium or magnesium or both was causing the symptoms. The roots resembled those of plants growing with insufficient calcium. The symptoms exhibited by the tops, however, did not resemble those of calcium or magnesium deficiency insofar as they were known. A macrochemical test of the soil was made. The soil colloid was found to be approximately 87 per cent calcium and 2 per cent magnesium saturated. The calcium content in the 6⅔-inch acre depth was estimated to be equivalent to 7 tons of ground limestone. The potassium content was equivalent to 3.4 tons of muriate of potash.

Soil was taken from the poorest section of the beds, placed in 8-inch pots, and treated with individual salts. A cucumber plant was set in each pot. The amount of material applied, consisting of individual salts in each case, was equivalent to what would be found in a ton of 5–8–7 fertilizer or a ton of hydrated dolomite. The pots were watered just enough to prevent wilting.

Data from the pot cultures showed that soluble calcium salts gave a normal type of growth when compared with that of the injured plants grown in soil with no treatment. Calcium nitrate and calcium chloride were more effective than calcium sulfate. Plants grown with additional superphosphate, indicated some correction. It was later noticeable that calcium sulfate and superphosphate were more effective when the soil was kept more freely watered.

[5] The water used in this range came from wells and contained considerable sodium chloride and some calcium chloride. Records showed a pH reaction several years back of 7.6 to 8.4. The water was later taken from a brook, and a fertilizer mixture of ammonium sulfate, potassium sulfate, and superphosphate was used to lower the pH. This treatment had lowered the pH when the soil was examined for this experiment.

Plants grown with sodium or potassium salts gave no response or were injured when the sulfate ion served as the carrier. This injury was very characteristic of that found in the greenhouse.

Roots on plants supplied with lime were very extensive and vigorous and, instead of growing toward the outside of the pot and between the walls of the pot and soil, as was the case where more soluble salts were applied, they were distributed all through the soil to the extent that the soil crumbled to pieces instead of coming out in a solid ball when the pots were dumped. This indicated a difference in aeration of the soil.

From these results it was apparent that the correction was brought about by the addition or greater availability of calcium, as is shown by the addition of potassium sulfate or chloride. The sulfate tended to decrease calcium availability, thereby producing a low calcium-high potassium condition to the extent that the potassium prevented calcium from being absorbed by the plants, as has been suggested by Lundgårdh (3). Apparently calcium sulfate was not sufficiently soluble in soils with low moisture content. The beneficial effect of calcium nitrate endured for 3 to 4 weeks, and repeated applications had to be made.

The relationship between calcium and potassium was repeated in sand culture. It was found that as long as calcium was maintained at a high level (0.0090 p.v.m. conc. of 1 atmos. solution) it was impossible to injure the growth. If, however, the calcium concentration was maintained at a low level (0.0014 p.v.m. conc.) the effect of large amounts of potassium was to produce a type of growth that was not symptomatic of any particular deficiency but was soft and succulent, as though the plants were watery in texture, and the foliage had a yellowish green cast resembling plants that do not assimilate nitrate readily. A discussion of this whole problem, interpreting the relation of calcium to potassium has been given by Lundegårdh (3). As far as the effect of the relationship of calcium to potassium is concerned, these results agree with his observations.

As a result of these observations, a recommendation was made that 1 ton of dolomitic limestone be used per acre each year, unless the soil reaction test indicated otherwise, that calcium nitrate be used as the source of nitrogen and superphosphate as the source of phosphoric acid, and that potash be withheld until a test showed that it was needed. Spent mushroom manure, which added some potassium, was being used on the beds.

Two years after the treatment was recommended, the soil was examined. Crop growth was much improved, and the roots on both cucumbers and tomatoes were white and vigorous. A test showed the presence of sufficient available calcium and of potash in liberal quantities. The soil no longer baked when dry nor did it pack down so firmly as before. In the past, nematodes had been a problem and steam sterilization of the soil did not seem to eradicate the pest completely because it was difficult to get the hard clumps of soil heated through. At the present time, the soil heats through readily and thorough

eradication is effected with much less heating. The excessive heating and the poor aeration of the soil in the past undoubtedly contributed to the poor growth, but apparently the correction of this soil depended on one of the many functions of calcium directly in the soil and indirectly on the plant growth.

The similarity of growth of vegetable crops on the coastal plain soils of New Jersey to the growth of plants observed in greenhouse soils prompted further experiments in sand and soil pot cultures to determine, if possible, how the chemical composition of the plant was changed by different ratios of available calcium to other cations. Some results on sodium are presented at this time.

TABLE 1

Partial volume molecular concentration of nutrient solutions used in determining the relation of calcium-sodium ratio to the growth of the tomato

SOLUTION*	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	$NaNO_3$†	$CaCl_2$	NaCl
A, 3 atmos.	0 0045	0 0045	0 0405	0 0045	. . . . .	. . . . . .
B, 3 atmos.	0 0045	0 0045	0 0225	0 0225	. . . . .	. . . .
C, 3 atmos.	0 0045	0 0045	0 0045	0 0405	. . . . . .	. . . .
D, 1.75 atmos.	0 0045	0 0045	0 0203	0 0023	. . . . . .	. . . . .
E, 1 75 atmos.	0 0045	0 0045	0 0113	0 0113	. . .	. . . . . .
F, 1.75 atmos.	0 0045	0 0045	0 0068	0 0156	. . .	. . . . . .
G, 1 75 atmos.	0 0045	0 0045	0 0023	0 0203	. . . . . .	. . . .
H, 1.5 atmos.	0 00675	0 00235	0 02025	0 00235	. . .	. . . . . .
I, 1.5 atmos.	0 00675	0 00235	0 00235	0 02025	. . .	. . . .
J, 1.5 atmos.	0 00675	0 00235	0.00235	. . . . .	0 02025	. . . . .
K, 1 5 atmos.	0 00675	0 00235	0 00235	. . . .	. . .	0 02025

* Concentrations are approximate and varied somewhat from values stated.

† The sodium nitrate furnished one-half as much nitrate as the calcium nitrate. This arrangement seemed necessary to keep calcium and sodium more comparable for purposes of this investigation. The same is true of the chloride content.

CALCIUM AND SODIUM RATIO AND GROWTH OF TOMATO IN SAND CULTURE

Studies on the effect of calcium and sodium ratio on plant growth were made on the tomato because of the various peculiar types of growth found in fields of canhouse tomatoes. A critical examination of the plants and of the soil showed that the problem was not one involving potassium because it was not being used in sufficiently large amounts to approach the concentrations which would produce abnormal growth of the foliage. Since growers, however, were using large amounts of sodium nitrate and very little calcium-carrying material, it seemed that a study on the effect of varying the calcium to sodium ratio on the growth of the tomato would be helpful as a guide to diagnosing peculiar growth conditions in the field even though the extreme ratios used in the pot cultures might not necessarily be encountered under field conditions.

Tomato plants were set in 3-gallon coffee urns in white, sifted, washed pit sand, and nutrients were applied by a continuous drip system. Eighteen-liter

inverted bottles served as reservoirs to supply the nutrients to five crocks, by means of asphalt-coated $\frac{3}{4}$-inch pipe and small pieces of capillary tubing at each crock to regulate the flow so that 3 liters were supplied each crock during 20 hours.

The nutrient solutions used are given in table 1. Two lots of plants were grown.

Plants in lot 1 were grown from February 5 to March 5 with solutions A, B, and C. Solutions having an osmotic concentration of approximately 3 atmospheres pressure were used because, during this time of the year when much cloudy weather prevails, plants grown in a more concentrated nutrient salt medium produce a less succulent growth which is more comparable to that of plants grown with abundant sunshine. The initial plants placed in the crocks were 6 to 7 inches high, were high in carbohydrates and low in nitrates, and had

TABLE 2

Dry matter in organs of tomato plants grown with nutrient solutions containing different ratios of calcium to sodium

SOLUTION	STEMS		PETIOLES		BLADES	
	Lower	Upper	Lower	Upper	Lower leaves	Upper leaves
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Iintial plants*	20 6	....	11.9	....	19 1	....
A, 9 Ca–1 Na	17.5	11 7	11 3	10 5	14.2	14 9
B, 5 Ca–5 Na	16.3	10 8	10 6	10 1	12.6	14.5
C, 1 Ca–9 Na	16.2	9 4	9.5	8.6	10.7	13.3
D, 9 Ca–1 Na	7.1		7 0		12.7	
E, 5 Ca–5 Na	6 5		6 3		11.2	
F, 3 Ca–7 Na	6 1		5 8		10.1	
G, 1 Ca–9 Na	6 0		5 4		9 3	

* Plants supplied with solutions A, B, and C were grown from February 5 to March 5. Plants grown with solution D, E, F, and G were grown from March 6 to April 3.

a gray-green appearance. A composite sample for dry matter determinations was made on blades, petioles, and stems. When the plants in the crocks were harvested, the upper and lower blades, petioles, and stems were analyzed separately.

Plants in lot 2 were grown with solutions D, E, F, and G. They were 2 inches tall, were comparatively soft and succulent, were dark green in color, and contained appreciable amounts of nitrates, but little starch. They were set in the crocks on March 6 and harvested on April 3. The solutions used had an approximate concentration of 1.75 atmospheres, inasmuch as light conditions were more favorable than for the group 1 plants.

The data on percentage of dry matter for both lots are given in table 2, carbohydrate fractions in table 4, and nitrogenous fractions for plants in lot 2 in table 3.

Regardless of the part of the plant examined, the data from plants grown with solutions A, B, C, (table 2) show a decrease in dry matter as the proportion of calcium is decreased and sodium is increased from a ratio of 9:1 to 1:9.[6] For example, the old stems decreased 1.3 per cent, the old petioles 1.8 per cent, and the old leaf blades 3.5 per cent, whereas the upper stems (younger tissue) decreased 2.3 per cent, the upper petioles 1.9 per cent, and the younger leaf blades 1.6 per cent. The younger plants, which were more succulent, grown

TABLE 3

Nitrogenous fractions of different parts of tomato plants grown with solutions in which the ratio of calcium to sodium was varied

Data expressed as percentage of green and dry matter

SOLUTION	TOTAL ORGANIC N		PROTEIN N		SOLUBLE ORGANIC N		NITRATE AND AMMONIUM N	
	Green	Dry	Green	Dry	Green	Dry	Green	Dry
Tomato stems								
D, 9 Ca–1 Na	0 1401	1 90	0 0801	1 05	0 0600	0 85	0 0800	1.12
E, 5 Ca–5 Na	0 1041	1 60	0 0757	1 16	0 0284	0 44	0 0876	1 35
F, 3 Ca–7 Na	0 0974	1 62	0 0670	1 12	0 0304	0 50	0 0856	1 40
G, 1 Ca–9 Na	0 0800	1 33	0 0552	0 92	0 0248	0 41	0 0856	1.43
Tomato leaf blades								
D, 9 Ca–1 Na	0 6230	4 84	0 5278	4 09	0 0952	0 75	0 0424	0 33
E, 5 Ca–5 Na	0 5277	4 67	0 4301	3 80	0 0976	0 87	0 0424	0 38
F, 3 Ca–7 Na	0 4789	4 75	0 3833	3 80	0 0956	0 95	0 0564	0 56
G, 1 Ca–9 Na	0 4229	4.55	0 3497	3 76	0 0732	0 79	0 0700	0.75
Tomato leaf petioles								
D	0 1248	1.76	0 1004	1 41	0 0244	0 35	0 1692	2 41
E	0 0984	1.52	0 0762	1 17	0 0222	0 35	0.1498	2.39
F	0.0926	1.49	0.0726	1 14	0 0200	0 35	0 1400	2 41
G	0 0630	1.17	0 0516	0.96	0 0114	0 21	0 1406	2 60

with solutions D, E, F, and G likewise showed similar trends, decreasing 1.1 per cent in the stems, 1.6 per cent in the petioles, and 3.4 per cent in the leaf blades.

[6] The ratios of calcium to sodium used in these experiments are extreme to facilitate interpretation of data for narrower ratios. It is doubtful whether these extremes would be encountered on coastal plain soils of New Jersey except in special temporary cases where the potential supply of calcium in the soil was low and a heavy application of nitrate of soda had been made. A number of cases have been identified in the culture of late celery where a heavy rain was preceded by a heavy application of sodium nitrate. Under such conditions leaching was sufficiently severe to carry the replaced calcium out of reach of the feeding roots. Complete calcium deficiency symptoms have been observed under such conditions.

In the plants grown in the low calcium-high sodium treatment[7] there was a consistent decrease in assimilated organic nitrogen (table 3) while the mineral nitrogen (nitrate and ammonium) showed no significant difference except in the leaf blades where the nitrate content increased while protein nitrogen decreased.

The carbohydrates decreased (table 4), although not so consistently as did the organic nitrogen, as the ratio of calcium to sodium changed from 9:1 to 1:9. The largest difference was between the 9:1 and the 5:5 ratios. The weight of the plants in the various groups was very similar. The high calcium

TABLE 4

Carbohydrate fractions in the blades and stems of tomato plants grown with nutrient solutions in which different proportions of calcium to sodium ions were present

Data expressed as percentage of green matter

SOLUTION	REDUCING SUGAR		TOTAL SUGAR		STARCH		HEMICELLULOSE	
	Blades	Items	Blades	Stems	Blades	Stems	Blades	Stems
Initial plants	1 008	1 778	1 104	2 221	6 156	3.121	0 287	0 226
Upper blades and stems								
A, 9 Ca–1 Na	0 675	0 679	1 062	1 203	0 700	0 220	0 206	0 296
B, 5 Ca–5 Na	0 674	0 524	0 841	0 967	0 384	0 178	0 186	0 216
C, 1 Ca–9 Na	0 359	0 531	0 804	0 919	0 297	0 160	0 219	0.244
Lower blades and stems								
A	0 395	0 670	0 618	1 642	0 366	0 670	0 182	0 294
B	0 390	0 579	0 567	1 549	0 199	0 693	0 130	0 290
C	0 233	0 612	0 399	1 575	0 171	0 483	0 139	0 421
Total blades and stems								
D, 9 Ca–1 Na	0 340	0 265	0 692	0 414	0 296	0 085	0 152	0 226
E, 5 Ca–5 Na	0 196	0 171	0 491	0 343	0 146	0 093	0 146	0 190
F, 3 Ca–7 Na	0 076	0 177	0 136	0 262	0 064	0 054	0 089	0.154
G, 1 Ca–9 Na	0 086	0 183	0 140	0.260	0 140	0 063	0 049	0 141

plants were more uniformly dark green in color than the high sodium plants. The general appearance of the plants indicated a difference in concentration of soluble organic nitrogen. Even though the high and low calcium soultions contained different amounts of nitrate (made necessary by the substitution of monovalent sodium for divalent calcium) the concentration of nitrate in the plants was similar (table 3).

[7] The methods were the same as those used for chemical analysis of tissue described in the New Jersey Agricultural Experiment Station Bulletin 547, but modified to include the method of extraction suggested by Davidson et al. in *Plant Physiology* 9: 817–822.

CALCIUM: SODIUM RATIO AND GROWTH OF TOMATO WITH HIGH AND LOW NITRATE SUPPLY

Two lots of plants, each consisting of 60 crocks with 3 tomatoes in each, were grown in sand culture, one with solution D and the other with solution G (table 1). The plan was to shift these to other solutions when the plants were 12 to 15 inches high. Before the plants could be shifted, however, a mosaic disease made its appearance and killed the plants in 55 of the crocks supplied with solution G (high sodium) but did only slight damage to the plants grown in solution D (low sodium). Although this was not part of the experiment, it was a good indication that these experiments were producing a type of growth which was similar to, and fully as susceptible to weather conditions and disease as, that of many tomato plants growing in the fields which were low in available calcium. The watery, light green, succulent growth of the high sodium plants grown with solution G and the darker green, less succulent plants grown with the high calcium solution D, even though of the same size, were characteristic of types of growth associated with good and poor growing conditions in the field.

The experiment was repeated. Although the tomato plants in the two groups were very similar to the plants in the two previous groups, they grew free of mosaic. Two 4-inch seedling plants comparatively soft and succulent and dark green were placed in each of the crocks on January 20, one-half being supplied with solution H and the other half with solution I (table 1), until March 1 when they were 12 to 14 inches high. At this time the plants receiving solution H (high calcium) were dark green, succulent, and growing rapidly. The other plants grown with solution I (high sodium nitrate) were slightly larger but lighter green and seemed to be more succulent than the calcium group. They wilted more easily when the day temperature increased as a result of bright sunshine. On March 2 the 120 plants grown with solution H were divided into four groups. Group 1 was continued with the same solution; group 2 was given solution I (high sodium nitrate); group 3 was given solution J (high calcium chloride in place of calcium nitrate); and group 4 was given solution K (high sodium chloride).

The 120 plants grown with solution I (high sodium nitrate) were likewise divided and treated as the previous group.

The plants were harvested on March 11, 9 days after being shifted to different solutions. The short interval elapsing between the shifting of solutions and harvesting was necessary to prevent excessive hardening due to accumulation of carbohydrates in the low nitrate groups. This accumulation of carbohydrates overshadowed the assimilation of the nitrate ion and consequently tended to mask the cation effect. Only the stems were harvested and analyzed. They were divided into 10 inches of base and 6 to 10 inches of tip of stem tissue. The dry matter and the nitrogenous fractions are shown in table 5.

There was very little difference in percentage of dry matter in the base of

the stems. In the upper portion of the stems, the results agree with those in table 2 in that there was 9.8 per cent dry matter in plants grown with sodium nitrate as compared with 11.0 per cent in those grown with calcium nitrate. The plants in the latter treatment, however, did not decrease in dry matter when shifted to sodium nitrate, as was expected; but the sodium nitrate plants did increase in dry matter when shifted to calcium nitrate, as was expected. When either group was shifted to the low nitrate solutions, carbohydrate accumulation apparently increased the dry matter still further. This increase was accentuated more by sodium chloride than by calcium chloride, apparently because nitrate assimilation is at a lower level where sodium is increased. This permits carbohydrates to accumulate more rapidly than where calcium is maintained at a higher level.

TABLE 5

Dry matter and nitrogenous fractions in the lower and upper stems of tomato plants after being shifted from one nutrient solution to another

SOLUTION* CHANGE	DRY MATTER		NITRATE N		SOLUBLE ORGANIC		PROTEIN N		TOTAL ORGANIC N	
	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem	Lower stem	Upper stem
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
H to H	7 0	11 0	0 0817	0 0866	0 0829	0 1034	0 0828	0 1147	0 1757	0 2181
H to I	7 0	11 5	0 0821	0 0807	0 0503	0 0901	0 0888	0 1065	0 1391	0 1966
H to J	7 2	12 1	0 0369	0 0389	0 0475	0 0778	0 0905	0.0976	0 1380	0 1754
H to K	7 2	12 3	0 0457	0 0331	0 0563	0 0917	0.0743	0 1027	0 1306	0 1944
I to I	7 0	9 8	0 0739	0 0807	0 0553	0 0653	0 0805	0 0987	0 1358	0 1640
I to H	7 0	11 0	0 0866	0 0816	0 0734	0 1028	0 1081	0 1236	0 1815	0 2260
I to J	7 0	11 1	0 0428	0 0273	0 0502	0 0847	0 0750	0 1035	0 1252	0 1882
I to K	7 0	13 0	0 0321	0 0369	0 0657	0 0719	0 0814	0.1057	0 1471	0 1776

* Composition of nutrient solutions given in table 1 (H = high calcium nitrate, I = high sodium nitrate, J = high calcium chloride, K = high sodium chloride).

The data on nitrogenous fractions are in better agreement with those given in table 2 than are dry matter determinations. Soluble organic nitrogen was much higher in the calcium nitrate plants than in the sodium nitrate plants. This is shown by the comparison between groups H and I. Where H was shifted to I, amino acids decreased, but where the shift was made from solution I to H amino acids increased. This holds as long as there was sufficient nitrate nitrogen present to prevent carbohydrate accumulation. The difference in amino acids was greater when the plants were shifted from solution I to H than from solution H to I. Had the plants been grown for a longer period on the shifted solutions, it is probable that even the H to I (Ca to Na) shift would have shown a greater difference. The protein nitrogen shows similar trends, although the differences were not so great as in the more quickly assimilated amino acids.

DISCUSSION

The importance of calcium in the nutrition of plants has been shown by Prianischnikov (6) and by Nightingale et al. (5). The importance of the calcium ion as an antagonizer of all other cations necessary for plant growth has likewise been shown (7). The data and observations presented in tables 2 to 5

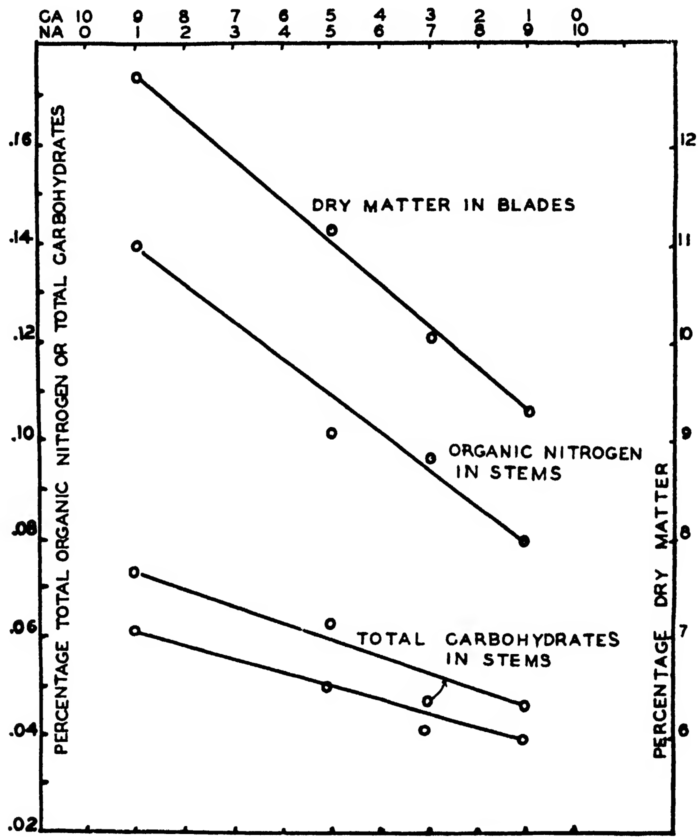

FIG. 1. EFFECT OF DIFFERENT RATIOS OF CALCIUM TO SODIUM ON DRY MATTER, ORGANIC NITROGEN, AND CARBOHYDRATE FRACTIONS IN TOMATO

inclusive further emphasize the importance of the calcium ion in regulating the growth of the plant.

Plants grown with an optimum supply of calcium apparently are dark green, possess a composition that is resistant to changes in environment, and present few physiological disorders because they assimilate nitrogen and carbon most rapidly. Even though the plants in the two groups, one grown with an optimum calcium supply and the other grown with a similar cation supply but comparatively low in calcium, made an equal volume of growth, but the quality

of growth was vastly different. As the calcium content in the nutrient solution decreased and the sodium increased, the tissue contained more water. Under conditions of rapid transpiration (lowered relative humidity) the high calcium plants showed no signs of wilting, whereas the low calcium plants wilted severely. In these cultures the ratio of calcium to sodium was not low enough to cause injury to the roots or to cause proteolysis as was suggested by Nightingale (5) in the case where calcium concentration was too low to support synthetic processes. In figure 1 are shown trends for dry matter, organic, nitrogenous, and carbohydrate fractions from plants grown with different amounts of calcium. A decrease in calcium is accompanied by a decrease in synthesized products. Were the curves extended to the point where there was no longer sufficient calcium to support synthetic processes, organic nitrogen would probably decrease but carbohydrates would increase rapidly for a short period until proteolysis disintegrated the carbohydrates more rapidly than they were being synthesized.

The difference in water content may be explained by the difference in affinity for water by calcium and sodium ions, as was pointed out by Flint (1). Mattson (4) has shown a difference in dispersion due to hydration of proteins when associated with calcium or sodium ions. It would seem possible that there are certain associations between sodium and proteins in the cell which would require the presence of more water. The dehydrating effect of the calcium ion introduced into such a system would tend to counteract or antagonize the effect of the sodium. In view of the fact that potassium has a similar effect to sodium, although to a smaller degree, it is entirely possible that one of the functions of potassium when absorbed is to counteract the dehydrating effect of an over-abundance of calcium.

From these results it would seem that a very important function of calcium is to hold the cell protoplasm in an equilibrium conducive to synthetic processes that build up proteins rapidly. This accumulation of proteins in turn gives stability to protoplasm, the amount of calcium needed for optimum synthesis being determined by the heredity of the plant. Thus plants which require a high pH likewise require a high calcium supply. A high soil pH brought about by ions other than calcium is not suitable because the physical properties when absorbed by the plant do not support synthetic processes as well. Thus the tomato which has a lower calcium requirement than the lima bean is less sensitive to soil conditions and tolerates a wider range of ratios than the lima bean, which makes very poor growth on soils that have a low calcium but otherwise high cation content. Many of our leguminous crops synthesize large amounts of proteins and also have a high calcium requirement.

A high proportion of protein to soluble organic nitrogen determines a certain quality (color and hardness) of growth. A high percentage of organic nitrogen means more dry matter and less water, or more substance to a given volume of growth. It is a common observation that spinach grown on a well-limed soil has a certain quality about it that prevents it from becoming yellow in

color and soft in texture after a heavy rain. The grower who does not use much lime complains of losing a crop of spinach because of a heavy rain. A comparison of spinach from a well-limed and a poorly limed field shows the difference in texture. Celery will wilt, turn yellow, and die following a heavy rain on soil low in calcium but freely supplied with other cations. The importance of the ratio of calcium to other cations, which probably is the cause of many of our nutritional problems on the coastal plain soils, cannot be over-estimated. The effect it has on the quality of growth makes it possible to blame much of our quality problems on the lack of available calcium (changing of ratio of ions) in the soil solution, particularly since its availability may vary between conditions of drought and abundant rainfall.

From the results presented in the discussion on the relation of the calcium to sodium and potassium ion it is safe to assume that even though sodium is not important as a nutrient for plant growth it probably has a marked effect on plant growth and is worthy of serious consideration in our fertilizer practices. It also offers a clue to the advantages and disadvantages of different nitrate carriers on different types or soils containing different proportions of cations.

SUMMARY

Soil and sand culture experiments are reported which show that soils may have a satisfactory reaction for crop growth but have too little available calcium for optimum synthesis of protein and carbohydrate materials.

An abundance of potassium may prevent the absorption of sufficient calcium on media low in calcium.

Plants grown with a ratio of 1 part calcium to 9 parts of sodium had from 1 to 3 per cent less dry matter than did plants grown with 9 parts of calcium and 1 part of sodium.

Associated with this decrease in dry matter, there was a poorer type of growth, which made plants more susceptible to wilting, to certain constitutional disorders, and to severe injury during periods of drought or heavy rainfall.

The ratio of available calcium to potassium or sodium was extremely important for germination of seed and growth of vegetable crops on coastal plain soils.

It is suggested that the effect of cations on growth is due to the hydration of protoplasm induced by different cations.

Nitrate-carrying salts apparently have other functions in the plant than merely supplying the necessary nitrogen for growth, a fact worthy of consideration in fertilizer practices.

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DEFICIENCY CHLOROSES IN CITRUS

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It is most difficult in the field to understand the causes of some physiological diseases of citrus that manifest themselves as chloroses in the leaves. A lack of iron is usually considered the cause of chlorosis when, in fact, there are chloroses that result from any number of different causes. The present paper does not deal with iron chlorosis but rather points out the symptoms of chlorosis that result from other so-called deficiencies such as manganese, sulfate, and magnesium; it is recognized, however, that there are many more types of chlorosis, such as calcium chlorosis (6) and zinc chlorosis.

The factors in these chloroses usually are most involved, and every clue may become a valuable aid in the diagnosis. In some cases a basis may be obtained by comparison of field data with the growth of citrus in solution or sand cultures under controlled conditions.

Figure 1, plate 1, illustrates the excellent growth of citrus roots of budded Valencia orange trees when grown in glazed crocks of 12-gallon capacity and when supplied with the nutrient salts necessary for healthy growth.

With the proper technique, budded citrus trees also may be grown in distilled water to which is added certain chemically pure inorganic salts (3). Figure 2, plate 1, shows the growth of budded Valencia orange trees in such solution cultures in 10-gallon acid carboys (neck portion removed). Aeration of deep solutions is necessary for healthy growth over any extended period. These methods of growing citrus under artificially controlled conditions were used in the present study of manganese, sulfate, and magnesium chloroses.

MANGANESE CHLOROSIS

The effects of a deficiency of manganese in the growth of some varieties of citrus have already been described (3). By means of solution cultures with budded Valencia orange trees and the use of a manganese-free iron supply it was possible to produce trees such as shown in figure 2, plate 1, with leaves bearing symptoms of chlorosis as a result of manganese deficiency. It should be pointed out that chlorosis in citrus may also be brought about by an excess of manganese (4).

Figure 1*A* and *B*, plate 2, illustrates the chlorosis in Valencia orange leaves produced in solution cultures by means of a manganese deficiency. The chlorotic spots may be small as in *A* or large as in *B*. The same type of chlorotic

spotting (pl. 2, fig. 1*C*) frequently is seen in the leaves of trees grown in soil containing considerable calcium carbonate. Steinberg (8) has shown that calcium carbonate may be utilized in solutions as a means of removing the last traces of the heavy metals, including manganese.

SULFATE CHLOROSIS

There are no known instances of chlorosis due to a sulfate deficiency occurring in the citrus districts of California. In order to know the appearance of the chlorosis that results from a sulfate deficiency, budded Valencia orange trees were grown for several years in 12-gallon glazed earthenware crocks filled with pure silica sand. The culture solution employed (3) was the same as that used for the control cultures (the roots of one of which is shown in figure 1, plate 1) except that nitrate was substituted for sulfate.

Figure 2, plate 2, shows the chlorotic appearance of the leaves. The total sulfur content in the dry matter of the various portions of these trees was compared with that in the dry matter of corresponding portions of trees of the same age grown in an adjacent nursery. The results are given in table 1.

TABLE 1

Total sulfur as percentage of dry matter

PORTION OF TREE	TREES GROWN IN PURE SILICA SAND WITHOUT SULFATE	TREES GROWN IN SOIL CONTAINING SULFATE
New mature leaves	0 048	0 233
New mature twigs	0 014	0.029
Trunk scion bark	0 036	0.017
Trunk root bark	0 031	0.077
Young rootlets	0.066	0 079

The chlorotic leaves of sulfate-deficient trees in sand cultures contained about one-fifth the percentage of total sulfur found in leaves of healthy trees grown in soil. The results show that sulfate-deficiency in citrus brings about a chlorosis in the leaves.

MAGNESIUM CHLOROSIS

Magnesium deficiency and the chlorosis caused by it have received most consideration in connection with the growth of tobacco and other crops (1) on sandy soils of eastern United States. A chlorosis of citrus due to a magnesium deficiency recently was described by Parbery (5) but is not illustrated. This physiological disease occurs in New South Wales. Its description agrees very well with that of the symptoms artificially produced (7) by a magnesium deficiency in pure silica sand cultures. Figure 1, plate 3, illustrates the chlorosis in Valencia orange leaves when the trees were grown in pure silica sand with a

culture solution lacking magnesium (7). As the chlorosis advances the bronzing effect on the leaf color increases.

Recently, chlorotic leaves were found on trees in southern California in locations close to the irrigation line; it is apparent that the trees have been over-watered at times with the loss of soluble magnesium as a possibility. Figure 2, plate 3, illustrates the symptoms of chlorosis found in the leaves of these trees. They resemble very closely those produced (7) in sand cultures when magnesium was deficient.

Chlorotic leaves from the trees from which those shown in figure 2, plate 3, were obtained, were found to contain 0.08 per cent of magnesium and 5.42 per cent of calcium in the dry matter, whereas healthy (control) leaves from trees of nearby unaffected groves contained 0.68 per cent of magnesium and 4.35 per cent of calcium respectively. Leaves from other affected trees contained 0.06 per cent of magnesium and 5.42 per cent of calcium in the dry matter, whereas control leaves from healthy trees in the same grove as the affected trees contained 0.30 per cent of magnesium and 5.46 per cent of calcium respectively.

Citrus bronzing in Florida (2) is considered to be a chlorosis that results from a magnesium deficiency.

It should be evident from these results that a chlorosis may be brought about by any of several causes. Whether or not iron within the tree is in some way affected in any or all of these chloroses is at present unknown. A knowledge of differences in the types of chlorosis symptoms produced physiologically in citrus leaves by a deficiency or an excess of certain elements may be of assistance in the diagnosis and control of chlorosis diseases.

SUMMARY

A study was made of the chloroses brought about in citrus trees by a deficiency of manganese, sulfate, and magnesium. Sand, soil, and solution cultures were used as media in which the trees were grown.

Manganese deficiency in orange trees was accompanied by a chlorotic spotting of the leaves. Such symptoms have been noted frequently in the field under conditions of excessive calcium carbonate.

The chlorosis that results from a deficiency of sulfate requires considerable time for the symptoms to become manifest and consists of a general yellowing of the leaf with the veins remaining green until the chlorosis becomes extreme.

When a deficiency of magnesium affects citrus leaves, the chlorosis at first occurs as a yellow stripe on each side of the dark green midrib. Later the leaves assume a bronze color.

A deficiency of any of these three elements in citrus is accompanied by a chlorosis which is more or less characteristic for the deficient element. A knowledge of the symptoms corresponding to certain deficiencies is of help in the diagnosis of physiological diseases in the field.

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PLATE 1

Fig. 1. Root system of a Valencia orange tree grown in pure silica sand in a glazed earthenware crock of 12-gallon capacity. Distilled water containing chemically pure salts (3) was used to maintain healthy growth.

Fig. 2. Growth of a budded Valencia orange tree in an aerated solution culture (3) in which phosphate was present for only a few days prior to the renewal of the solution and in which 5 to 10 p.p.m. of aluminum was present when phosphate was absent. This is the largest citrus tree grown to date in a solution culture. The ruler placed next to the trunk is 1 foot long.

Fig. 1

Fig. 2

PLATE 2

FIG. 1. Chlorosis as a result of manganese deficiency in Valencia orange leaves. *A*, dorsal (upper) surface of leaf from tree grown in a controlled culture solution lacking manganese. *B*, translucent spots as seen from the ventral (lower) surface of a leaf obtained from a tree grown in a manganese-deficient culture solution. *C*, leaves from trees in the field where the soil contained large amounts of calcium carbonate.

FIG. 2. Chlorosis in Valencia orange leaves from trees grown in pure silica sand to which was applied a culture solution lacking sulfate.

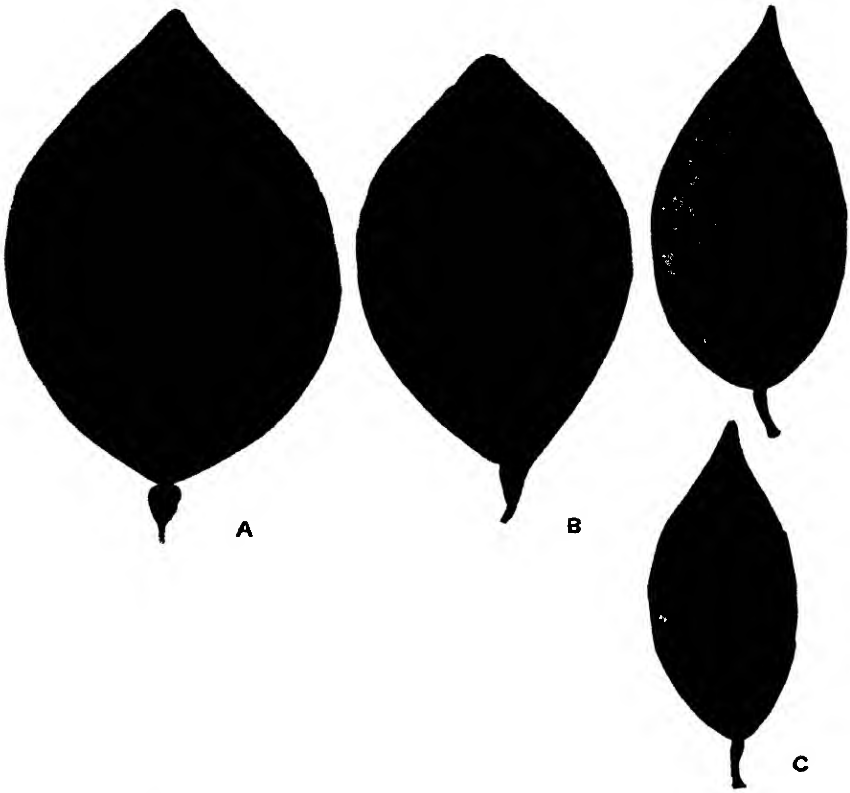

Fig. 1

Fig. 2

PLATE 3

FIG. 1. Chlorosis of Valencia orange leaves obtained from trees grown in pure silica sand to which a culture solution lacking magnesium was applied. Note the increase in the bronzing effect as the chlorosis becomes more severe

FIG. 2 Chlorosis of Valencia orange leaves obtained from trees in the field in locations close to the irrigation line Overwatering may be responsible for the loss of magnesium and the production of magnesium-deficiency chlorosis.

FIG. 1

FIG. 2

THE TOLERANCE OF NITRATE BY PURE CULTURES OF AZOTOBACTER[1]

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There are numerous references in the bacteriological literature to both a beneficial and an injurious effect of nitrates upon Azotobacter. A detailed discussion of these apparently conflicting data is not necessary in this connection. Suffice it to say that a general survey of the available information has led to the conclusion that in relatively low concentrations nitrates are beneficial, or, at least, not injurious. On the other hand, if the concentration is relatively high an injurious effect is produced, not, however, necessarily due to a directly toxic effect upon the Azotobacter cell but possibly, in soil at least, to an indirect effect upon some environmental factor.

In recent bacteriological and chemical studies of soil from "fertile" and "nonfertile"[2] areas taken in close proximity, "the density of the Azotobacter population"[3] was repeatedly observed to be greater in the less productive soil. At the same time the concentration of nitrates was almost invariably greater in the more productive soil. Fertile soils of the type studied produced, as an average for the 5 years during which yields were recorded, 2.4 times as much grain and 3.0 times as much protein as did the nonfertile soil. Elsewhere[4] data are being presented which show that this difference in productivity can be explained wholly on the basis of differences in available nitrogen. Inasmuch as this inverse relationship between the Azotobacter population and the nitrate content was so consistently observed, the inference might be drawn that the lower Azotobacter population resulted from the higher nitrate content, but the

[1] Contribution No. 170, department of bacteriology, Kansas Agricultural Experiment Station.

[2] The term "fertile" as here used has reference to the well-defined, darker green and more vigorously growing areas, usually 2 to 3 feet in diameter, described and pictured in "The rôle of nitrogen in the production of spots in wheat fields," by P. L. Gainey and M. C. Sewell, *Jour. Agr. Res.* 45: 129–148 (1932). "Nonfertile" has reference to the area immediately surrounding the "fertile" spot. The expressions "fertile" and "nonfertile" should be taken literally only when any particular pair of samples is compared. The "nonfertile" soil of one field may have been actually more productive than the "fertile" soil of another field.

[3] This term is used as employed by S. Winogradsky and Mme. J. Ziemiecka in "Etudes sur la microbiologie du sol. Sur le pouvoir fixateur des terres," *Ann. Inst. Pasteur Mem.* 3, 42: 36–62 (1918).

[4] Gainey, P. L., Sewell, M. C., and Meyers, H. E. Nitrogen—the major cause in the production of spotted wheat fields. Kans. Agr. Exp. Sta. Tech. Bul. (In press).

actual observed concentration of nitrates in the more productive samples was frequently so low as to cast doubt upon such an assumption. It should be noted, however, that there is no unanimity of opinion expressed in the literature as to the concentration of nitrates necessary to cause injury to Azotobacter, and the recorded minimum injurious concentrations vary widely. In view of the well-established fact that the organisms of this group vary markedly in many of their characteristics, it seemed plausible that the variations observed

TABLE 1

Nitrate content and density of Azotobacter flora in fertile and nonfertile soil

1930					1931				
Soil Number	NO_3 in soil		Colonies Azotobacter		Soil number	NO_3 in soil		Colonies Azotobacter	
	Nonfertile	Fertile	Nonfertile	Fertile		Nonfertile	Fertile	Nonfertile	Fertile
	p.p.m.	*p.p m.*				*p.p m*	*p.p.m.*		
2	Tr.	6 0	74	64	1	10 0	10 0	19	3
3	2.0	23.0	22	36*	5	9 7	50 7	19	10
4	0	11 0	110	121*	7	7.7	99 3	21	16
6	3 0	3 0	30	29	8	7.8	342 7	175	75
8	4 0	16 0	80	55	10	6 7	86 6	50	40
11	2 0	20 0	95	74	12	9 3	158 2	95	50
16	2 0	5 0	21	9	13	9 6	140 2	75	45
18	Tr.	38 0	263	190	14	4 5	10 5	90	18
19	Tr.	14 0	530	176	16	6 2	64 8	1	3*
21	0	10 0	48	29	17	16 5	22 3	5	1
23	Tr.	10 0	510	430	20	5 3	156 3	59	7
24	0	Tr.	34	2	25	4 8	25 1	35	45*
25	0	15 0	92	90	27	3 8	32 7	2	2
27	0	0	68	27	28	4 8	47 1	49	26
28	0	13 0	6	1	29	2 5	6 4	32	10
29	Tr.	Tr.	115	115	31	4 7	109 0	90	70
31	Tr.	Tr.	140	155*	32	3.1	24 4	105	105
35	0	Tr.	1000	550	33	3 9	21.8	3	2
37	Tr.	6 0	148	127	34	1 4	5 8	50	6
39	0	12 0	14	2	36	3 2	4.0	56	33
40	0	52 0	125	190*	40	3 0	5 9	160	155
43	0	Tr.	79	36					
Ave.	0 6	11.5	164	114		6.1	67.8	54	33

* Instances in which the colony count was higher in the fertile sample.

in tolerance to nitrates, even though the nitrate content was relatively low, might be explained on a basis of strain variation.

FIELD OBSERVATIONS

Differences in the density of the Azotobacter population were determined as suggested by Winogradsky,[5] except in the type of medium employed. Freshly

[5] Winogradsky, M. S. 1925 Etudes sur la microbiologie du sol. I. Sur la méthode. *Ann. Inst. Pasteur* 39: 299–354.

prepared poured plates of washed mannite agar were inoculated by sprinkling a weighed quantity of the soil over the surface and these were incubated at 28° to 30°C. The Azotobacter colonies developing were counted after 3 to 5 days.

In table 1 are presented the data relative to the number of Azotobacter colonies developing from, and the nitrate content of, the soils examined in 1930 and 1931. Only data from the soils examined which exhibited Azotobacter by this method are included. Azotobacter were not detected in either sample of many soils. It is evident from these data that even though differences in Azotobacter and in nitrate nitrogen contents are not marked, yet a fairly consistent inverse ration between the two can be noted.

Relative to the nitrate content, attention should be directed to the fact that wheat was growing on all these soils and that the samples were taken at about the time the grain was making its maximum growth, hence, a heavy draught was being made upon the soil's store of soluble nitrogen. Furthermore, at the same time the soil samples were taken, the above-surface plant growth was collected from a measured area, dried, weighed, and analyzed for total nitrogen. For the season of 1930, 4.8 and for 1931, 4.2 times as much nitrogen had been removed from the soil by the aerial growth on the fertile area as had been removed by the corresponding growth upon an equal nonfertile area. If it is assumed that this nitrogen was absorbed in the form of nitrates it is possible that variations in the concentrations were much more marked somewhat earlier than at the time the samples were taken. Furthermore, the source of the higher nitrate content, in case of the fertile areas, has been traced to the nitrification of urea in urine deposited by cattle grazing upon the growing grain during late fall and early spring. Since the samples studied were collected during a period beginning shortly after active growth started in the spring and ending before flowering of the plants, the existence of high nitrate contents must have been of short duration, at least in many instances. The low nitrate content, even of the fertile samples, for 1930 indicates the existence of unfavorable conditions for nitrate formation and hence its consumption by the growing plants as rapidly as formed.

The counts recorded in table 1 represent the colonies developing from the particles of soil contained in 1 or 0.5 gm. soil and do not represent individual organisms; hence, the relatively low counts. Because of the heavier growth of wheat on the fertile soil, the moisture content of the nonfertile sample was almost invariably slightly higher. Since the weighed inoculum was not corrected for moisture, the actual quantities of soil used as inoculum were slightly less in the case of the nonfertile soil. This condition and also the tendency of the more moist soil to form larger particles would result in colony counts lower than actually obtained in case of the nonfertile soil and would, to that extent, minimize observed differences between the fertile and the nonfertile samples.

Inasmuch as the soils were obtained from a wide variety of conditions and as only one pair of samples was collected from any one field, it was thought well to verify the variations in the Azotobacter count between fertile and non-

fertile soils by collecting a number of samples from the same field. In 1933, 26 pairs of samples were taken from the same field. The data relative to these samples are recorded in table 2.

Although the colony counts recorded in either table 1 or 2 are not markedly in favor of the less fertile of a pair of samples, they tend in that direction so consistently that there is little doubt of the existence of an actual difference. Of 69 samples here included only 9 gave higher counts in the more fertile of the pair.

TABLE 2

Density of Azotobacter flora in fertile and nonfertile samples of soil all from same field

SAMPLE NUMBER	COLONIES AZOTOBACTER		SAMPLE NUMBER	COLONIES AZOTOBACTER	
	Nonfertile	Fertile		Nonfertile	Fertile
1	92	3	15	27	26
2	167	160	16	112	93
3	106	92	17	64	38
4	169	70	18	110	154*
5	123	56	19	165	80
6	120	64	20	111	53
7	27	31*	21	47	5
8	107	88	22	59	28
9	98	38	23	87	75
10	188	153	24	132	117
11	210	170	25	94	74
12	3	2	26	101	84
13	5	8*			
14	66	45	Average....	100	70

* Instances in which the colony count from fertile samples exceeded that from the nonfertile.

$$\text{Ratio 1930 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1.44$$

$$\text{Ratio 1931 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1\ 64$$

$$\text{Ratio 1933 } \frac{\text{Nonfertile}}{\text{Fertile}} = 1.43$$

LABORATORY OBSERVATIONS

To gain information relative to the concentration of nitrates and the length of time necessary appreciably to affect the Azotobacter population of soils of the type under study, laboratory experiments were conducted on two different soils, S and X.

The soils were brought to the laboratory, passed through a 2-mm. sieve, and kept in moist condition for some time in order that more or less of a biological equilibrium might be reached under laboratory conditions. A sufficient number of samples were then prepared to permit of the analyses indicated in

tables 3 and 4. To these samples were added the indicated quantities of nitrate and urea nitrogen, along with sufficient water to bring the X samples to 50 per cent saturation (30 cc. per 100 gm. soil) and the S samples to 33 per cent saturation (27 cc. per 100 gm. soil). Larger quantities of moisture could not

TABLE 3

Influences of the addition of nitrate and urea nitrogen upon the Azotobacter colony count in soils S and X

NITROGEN ADDED TO SOIL			COLONY COUNT* OF AZOTOBACTER AFTER VARYING PERIODS OF TIME						
Form	Soil	Amount	0 wks.†	2 wks.	4 wks	8 wks.	12 wks.	16 wks.	20 wks.
		p.p.m.							
KNO_3	X	0	65	71	65	84	62	68	31
KNO_3	X	50	55	86	71	82	60	88	63
KNO_3	X	150	61	88	67	74	67	71	40
KNO_3	X	500	72	91	60	53	78	79	38
Urea	X	50	82	95	57	51	73	68	53
Urea	X	150	81	72	83	82	85	87	36
Urea	X	500	77	81	50	48	69	67	19
KNO_3	S	0	53	74	60	68	53	49	...
KNO_3	S	50	57	79	55	66	34	43	...
KNO_3	S	150	49	59	41	19	10	14	...
KNO_3	S	500	31	6	0	0	0	0	...
Urea	S	500	—	79	49	0	0	0	...

* Count from 3-day-old undiluted plates in case of soil X and diluted plates in case of soil S.
† Count one day after treatment.

TABLE 4

Influence of nitrate nitrogen upon the colony count of Azotobacter in soils S and X

NITROGEN ADDED TO SOIL AS KNO_3	SOIL	COLONY COUNT* OF AZOTOBACTER AFTER VARYING PERIODS OF TIME			
		0 wks.†	2 wks.	4 wks.	8 wks.
p.p.m. N					
0	X	26	21	34	14
500	X	23	14	18	9
1000	X	25	9	9	6
2000	X	18	9	5	1
0	S	61	79	40	26
500	S	87	9	7	2
1000	S	39	0	0	0
2000	S	32	1	0	0

* Count on diluted plates after 3 days of incubation.
† Sampled one day after treatment.

be employed in either case because of puddling. In these tablets are also recorded the Azotobacter density following various treatments after varying intervals of time. During incubation the moisture content was kept constant by frequent additions of water.

Because of the high initial Azotobacter content of these soils it was necessary to resort to some method of diluting before plating. This was accomplished by thoroughly mixing 1 part of the soil in question with 9 parts of an Azotobacter-free soil. The diluting soil had previously been passed through a 20-mesh sieve but retained by a 40-mesh sieve. This eliminated the finer particles, which apparently aided in the coalescing of colonies, and facilitated the formation of discrete colonies.

If the NO_3 ion is responsible for the decrease in the Azotobacter population here recorded, the question naturally arises as to how the decrease in Azotobacter is to be accounted for when nitrogen in the form of urea is added. Suffice it to say that all these soil samples were analyzed for nitrate nitrogen, and the urea nitrogen was found to be transformed into nitrate nitrogen very rapidly. The following data relative to the NO_3—N content of soil X after 4 weeks illustrate this:

Treatment p.p.m. N	*NO_3-N recovered p.p.m.*
0 as KNO_3	43.4
50 as KNO_3	114.6
150 as KNO_3	210 2
500 as KNO_3	559.6
50 as urea	103 7
150 as urea	233 7
500 as urea	576.8

It is evident from the data presented in table 3 that the injurious effect of the application of nitrogen was slightly delayed in the case of urea; this fact indicates the necessity for a sufficient lapse of time to enable nitrification to take place. Nitrification did not take place so rapidly in soil S as in soil X. Aside from this slight delay in accumulation, the nitrate contents of the samples treated with urea nitrogen were, on the average, as high as those treated with nitrate nitrogen.

The following three points seem worthy of special note in connection with the data recorded in tables 3 and 4:

There was a marked difference in the sensitivity to nitrates of the Azotobacter in the two soils, or else the environmental conditions were such as to render the organisms in soil S less resistant to injury.

A decrease in the Azotobacter density, as here determined, became evident in a relatively short period of time; there were some indications of such an effect within 24 hours and a very marked effect within 2 weeks.

The concentration of nitrates, expressed in parts per million soil, necessary to reduce appreciably the density of the Azotobacter population may vary in different soils but is certainly not very high for soil S under the conditions existing in these experiments.

PURE CULTURE STUDIES

A large number of cultures, all isolated from local soils, have been studied with reference to their tolerance of nitrates. Under suitable conditions all these cultures produced a typical black pigment and for this reason have been

tentatively classed as strains of *Azotobacter chroococcum*, though no effort has been made to verify this grouping. All isolations and purification of cultures were made on mannite agar to which no nitrogen was added. With the exception of the early cultivation of series I all cultures were grown on mannite agar slants, prepared from washed agar-agar (100 gm. powdered agar-agar suspended in 4 liters distilled water and the water renewed every day or two for a period of 2 weeks) to which the different nitrates were added to give the desired concentrations of NO_3—N. The washing process reduced the total nitrogen content of the agar from 25–30 p.p.m. to 5–8 p.p.m. Such an agar to which no nitrogen was added is designated as "nitrogen-free," and although this is technically incorrect the nitrogen present must have been in a highly complex insoluble form and, therefore, probably not readily available to Azotobacter. Cultures were transferred every 3 or 4 days and incubated at 28° to 30°C. Observations of growth and pigmentation were recorded after 3, 7, and sometimes 14 days. It required 2 to 4 days for cultures to attain their maximum visible growth, and much longer periods between transfers at high nitrate concentrations were decidedly injurious.

The composition of the nitrogen-free medium employed was as follows:

Dipotassium phosphate	2 50 gm.
Magnesium sulfate	0 20 gm.
Sodium chloride	0.20 gm.
Calcium chloride	0.05 gm.
Ferric chloride, 10 per cent solution	1 drop
Mannite	20.00 gm.
Agar-agar, washed	20 00 gm.
Distilled water	1000 cc.

After the medium was autoclaved to dissolve the agar-agar, the salts, a few drops of phenolphthalein indicator, and sufficient *N* NaOH to give a faint, but permanent, pink color were added. The reaction of such a medium upon standing was always acid to phenolphthalein and alkaline to brom-thymol blue, hence in the vicinity of pH 8.0, since sufficient buffering effect was produced by the phosphate to prevent any marked change in pH.

Series I. Series I consisted of 13 cultures isolated and grown on unwashed agar containing 0, 1, 10, 25, 50, 75, and 100 p.p.m. KNO_3—N for a period of 190 days. The only consistent differences observed were that cultures 8, 20, 40, and 36 were a little slow in developing on 100 p.p.m. KNO_3—N following the first few transfers, and that cultures 5 and 32 exhibited a similar retarded growth at 50 and 75 p.p.m. and failed to grow at 100 p.p.m. KNO_3—N when inoculated from the nitrogen-free medium but grew normally when inoculated from the 75 p.p.m. N medium. These are the only cultures that received a preliminary cultivation on unwashed agar.

After a period of 70 to 73 days on the unwashed agar medium each culture was transferred to washed agar containing the corresponding concentrations of KNO_3—N. They were continued on these media for periods of 116 to 123

days. The only consistent difference observed was that culture 40 grew poorly on the nitrogen-free medium for a short time.

These 13 cultures were left on nitrogen-free agar for a period of 7½ months, after which 9 were recultured and tested for purity. These, together with two new isolations, were transferred to washed agar containing 0, 100, 500, 1000,

TABLE 5

Effect of various concentrations of KNO_3–N upon the growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.									
	0	100	500	1000	3000		4000		5000	
	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
5	4x	4x	4x*	1x–3x	0	0	0	0	0	0
					1000	0	1000	0	1000	0
8	4x	4x	4x	4x	0	0	0	0	0	0
					1000	4x†	3000	1x–4x	3000	0
									4000	1x–4x
12A	4x	4x	4x	4x	0	4x	0	4x	0	4x
12B	4x	4x	4x	4x	0	4x	0	4x	0	0‡
									4000	4x
31	4x	4x	4x	4x	0	0	1000	0	1000	0
					1000	4x*				
36	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	1x–4x	4000	0
A	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	1x–4x	4000	0
EA	4x	4x	4x	4x	0	4x†	0	0	0	0
							3000	4x	3000	0
									4000	4x†
EB	4x	4x	4x	4x	0	4x†	0	0	0	0
							1000	0‡	1000	0‡
S	4x	4x	4x	4x	0	0	0	0	0	0
							1000	0	1000	0
X	4x	4x	4x	4x	0	4x†	0	1x–4x	0	0
									3000	0
									4000	1x–4x

* Markedly retarded.
† Some retardation.
‡ Died after few transfers.
(In this and all subsequent tables 4x = normal; 2x = one half normal growth; etc.)

3000, and in some instances 4000 and 5000, p.p.m. KNO_3—N. Because of difficulties sometimes encountered in inducing growth on the higher concentrations and also because of an accidental failure to transfer cultures regularly during one period of 4 weeks, at which time every culture completely lost its vigor at concentrations varying from 500 to 3000 p.p.m. N and had to be

started again from the N-free culture, there was considerable irregularity with respect to the time the different cultures were grown at various concentrations. Where repeated transfers from the N-free medium to higher concentrations failed to grow, some lower concentration was used as the source of inoculum. In this way normal growth of Azotobacter frequently could be obtained at much higher concentrations than when cultured only from the N-free culture. Also, in some instances where growth of a culture was very slight after 3 to 4 days and no perceptible growth appeared when the culture was transferred, if the original culture was allowed to grow for 7 to 10 days and then was transferred, growth might result. In a few instances fair or even normal growth would take place for a period of time at relatively high concentrations and then gradually die out. On other slants growth at high concentrations would be slow or feeble for a time, after which it became normal.

TABLE 6

Effect of different concentrations of $NaNO_3$—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATIONS OF NITROGEN, P.P.M.								
	0	500	1000	3000		4000		5000	
	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
S	4x	4x	4x	0	0				
				1000	1x–4x*				
X	4x	4x	4x	0	0†				
				1000	2x–4x				
12B	4x	4x	4x	0	4x	0	2x–4x	0	2x–4x
8	4x	4x	4x	0	4x				

* Grew irregularly for a few weeks then died.

† Died after a few transfers.

The term "normal" as here used has reference solely to the mass of visible growth occurring on agar slants, growth on the nitrogen-free agar being taken as standard. It might be suggested that morphologically many of the cultures could not be regarded as normal. Many irregular and bizarre forms were observed, particularly in the $Ca(NO_3)_2$ media and in higher concentrations of other forms of nitrate. The relative growth of these cultures on agar containing varying concentrations of NO_3—N is recorded in table 5.

It is worthy of note that culture 5 which would not grow on agar containing 100 p.p.m. NO_3—N originally was finally induced to grow normally at 500 and irregularly at 1000 p.p.m. NO_3—N.

In order to obtain evidence that the injurious effect of nitrates is due to the NO_3 ion and not to the cation, other salts of HNO_3, including $NaNO_3$, $Ca(NO_3)_2$, and NH_4NO_3, were substituted for KNO_3. The data from series I grown in the

presence of these forms of nitrogen are presented in table 6, 7, and 8. From these data the following points appear worthy of special attention:

A marked variation in the tolerance of different strains of Azotobacter to the nitrate ion is evident. For example, cultures 5 and S failed to grow at 3000 p.p.m. NO_3—N, the former

TABLE 7

Effect of different concentrations of $Ca(NO_3)_2$—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.					
	0	500	1000		3000	
	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth
S	4x	4x	0	0	0	0
			500	0*	1000	0
X	4x	1x–4x	0	0	0	0
			500	0*	1000	0
EA	4x	4x	0	0*	0	0
			500	1x‡	500	0
12B	4x	4x†	0	0	0	0
			500	0*	500	0
8	4x	0*	0			

* Died after a few transfers.
† Retarded following first few transfers.
‡ Growth questionable.

TABLE 8

Effect of different concentrations of NH_4NO_3—N upon growth of pure cultures of Azotobacter—series I

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.						
	0	250	500	1000	1500	3000	
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth
S	4x	4x	4x	4x	2x–4x	0	0
						1000	0
X	4x	4x	4x	4x	4x	0	1x–4x*
12B	4x	4x	4x	4x	4x	0	0
						1500	1x–3x
8	4x	4x	4x	4x	1x–4x	0	1x–3x*

* Died after a few weeks.

appearing especially susceptible to injury. On the other hand, cultures 8, 12A, 12B, EA, and X all grew at 5000 p.p.m. NO_3—N, 12A growing normally at this concentration even when brought directly from the N-free medium.

Many strains of Azotobacter can be induced, by gradual adaptation, to grow normally in concentrations of nitrates in which they otherwise will not grow.

TABLE 9

Effect of different concentrations of KNO_3—N upon the growth of pure cultures of Azotobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.											
	0	100	500	1000	2000	4000		4500		5000		
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth	
BB	4x	4x	4x	4x	4x	0	4x*	4000	4x	4000	4x	
IIA	4x	4x	4x	4x	4x	0†	1x–4x*	4000	0	4000	0	
IIB	4x	4x	4x	4x	4x	0†	4x*	4000	1x–4x	4000	1x–4x	
IIC	4x	4x	4x	4x	4x	0†	1x–4x	4000	1x–4x	4000	1x–4x	
IIIC	4x	4x	4x	4x	4x	0	1x–4x	4000	1x–4x	4000	1x–4x	
IIID	4x	4x	4x	4x	4x	0†	1x–4x*	4000	1x–4x	4000	0‡	
IIIE	4x	4x	4x	4x	4x	0	1x–4x*	4000	0‡	4000	0‡	
IIIF	4x	4x	4x	4x	4x	0	1x–4x	4000	0‡	4000	0‡	
IID	4x	4x	4x	4x	4x*	0†	1x–4x*	4000	0‡	4000	0‡	
IIE	4x	4x	4x	4x	4x	0	1x–4x*	4000	1x–4x	4000	1x–4x	
IIIA	4x	4x	4x	4x	4x	0†	1x–4x*	4000	1x–4x	4000	1x–4x	
IIIB	4x	4x	4x	4x	4x	0	1x–4x*	4000	1x–4x	4000	1x–4x	

* Growth retarded following first few transfers.

† Transferred from 0 after 3–4 days, growth failed; transferred from 0 after 10 days, growth took place.

‡ Died after a few transfers.

TABLE 10

Effect of different concentrations of $Ca(NO_3)_2$—N upon the growth of pure cultures of Azotobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.					
	0	100	500	1000	2000	4000
	Growth	Growth	Growth	Growth	Growth	Growth
BB	4x	4x	4x	2x–4x	2x–3x	0
IIA	4x	4x	4x	2x–4x	1x–4x	0
IIB	4x	4x	2x–4x	2x–4x	2x–4x	0
IIC	4x	4x	2x–4x	1x–4x	1x–2x	0
IIIC	4x	4x	2x–4x	1x–4x	0x–1x	0
IIID	4x	4x	4x	4x	0x–1x	0
IIIE	4x	4x	2x–4x	2x–4x	1x–4x	0
IIIF	4x	4x	1x–4x	1x–4x	0x–4x	0
IID	4x	4x	3x–4x	2x–4x	1x–4x	0
IIE	4x	..	2x–4x	2x–4x	1x–3x	0
IIIA	4x	4x	2x–4x	2x–4x	1x–3x	0
IIIB	4x	4x	2x–4x	1x–4x	0x–1x	0

TABLE 11

Effect of different concentrations of NH_4NO_3—N upon the growth of pure cultures of Actobacter—series II

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.													
	0	50 (100)*	250 (500)	500 (1000)	1000 (2000)	2000 (4000)	2500 (5000)		3000 (6000)		3500 (7000		4000 (8000	
	Growth	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth	Inoculated from	Growth
BB	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	4x	0	0
													1000	0
													2000	0†
IIA	4x	4x	4x	4x	4x	4x	2000	4x	2000	1x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIB	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	0†	0	0
													1000	0
													2000	0†
IIC	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	4x	0	0
													1000	0
													2000	0†
IIIC	4x	4x	4x	4x	4x	4x	2000	2x–4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIID	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIE	4x	4x	4x	4x	4x	4x	2000	4x	2000	4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIF	4x	4x	4x	4x	4x	4x	2000	1x–4x	2000	1x–4x	2000	0	0	0
													1000	0
													2000	0†
IID	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–4x	2000	2x–4x	0	0
													1000	0
													2000	0†
IIE	4x	4x	4x	4x	4x	4x	2000	3x–4x	2000	2x–4x	2000	0†	0	0
													1000	0
													2000	0†
IIIA	4x	4x	4x	4x	4x	4x	2000	2x–4x	2000	2x–4x	2000	1x–4x	0	0
													1000	0
													2000	0†
IIIB	4x	4x	4x	4x	4x	4x	2000	4x	2000	2x–3x	2000	1x–4x	0	0
													1000	0
													2000	0†

* Values in parenthesis are total nitrogen.

† Died after a few weeks.

There is some indication that the sensitivity or tolerance to nitrate of pure cultures is associated with a similar sensitivity or tolerance of mixed cultures in soil. Cultures S and X were isolated from the corresponding soils. Soil S has already been described as containing a much more sensitive Azotobacter flora than X. A similar variation was observed in the pure cultures S and X.

No significant difference was noted as to the effect of the various nitrates except the apparently more toxic action of $Ca(NO_3)_2$. This difference is believed to be due, however, to the removal of phosphorus from solution in the presence of the high calcium content rather than to the nitrate ion.

TABLE 12

Effect of different concentrations of KNO_3—N upon the growth of pure cultures of Azotobacter—series III

CULTURE NUMBER	CONCENTRATION OF NITROGEN, P.P.M.						
	0	100	500	1000	2000	4000	
	Growth	Growth	Growth	Growth	Growth	Inoculated from	Growth
1	4x	4x	4x	4x	4x	0	1x–4x
2	4x	4x	4x	4x	4x	0	1x–4x
3	4x	4x	4x	4x	4x	0	0
						2000	0
4	4x	4x	4x	4x	2x–4x	0	0
						2000	0*
5	4x	4x	4x	4x	4x	0	1x–4x
6	4x	4x	4x	4x	4x	0	4x
7	4x	4x	4x	4x	4x	0	1x–4x†
8	4x	4x	4x	4x	4x	0	1x–4x†
9	4x	4x	4x	4x	4x	0	1x–4x
10	4x	4x	4x	4x	4x	0	0
						2000	0
11	4x	4x	4x	4x	2x–4x	0	0
						2000	0
12	4x	4x	4x	4x	4x	0	2x–4x†
13	4x	4x	4x	4x	4x	0	1x–4x†
14	4x	4x	4x	4x	2x–4x	0	0
						2000	2x–4x
15	4x	4x	4x	4x	4x	0	4x†

* Died after a few weeks.

† Growth retarded following first few transfers.

Series II. Series II consisted of 12 cultures all isolated, purified, and immediately transferred to varying concentrations of nitrate nitrogen in the form of KNO_3, NH_4NO_3, and $Ca(NO_3)_2$. After a period of 18 weeks on concentrations up to and including 4000 p.p.m. KNO_3—N, efforts were made to grow the cultures at 4500 and 5000 p.p.m. N by transferring them from the 4000 p.p.m. culture to the higher concentrations. Similarly, after 16 weeks on concentrations up to and including 4000 p.p.m. NH_4NO_3—N, efforts were made to grow

cultures at 5000, 6000, 7000, and 8000 p.p.m. of the same forms of nitrogen. Summaries of observations as to growth are recorded in tables 9, 10, and 11.

In this series, as in the first series, differences in tolerance to NO_3 are evident, though perhaps not so marked as in the former series. Also the cultures all appear to be less tolerant of NO_3 in the form of $Ca(NO_3)_2$ than other forms; however, as already suggested this difference was probably due to the lack of available phosphorus. Here again, by gradual adaptation, cultures could be induced to grow at much higher concentrations of NO_3 than they would tolerate normally.

Series III. A third group of cultures was isolated, purified, and immediately cultured upon agar containing varying concentrations of NO_3—N. These were grown for a period of 95 days, during which time efforts to induce the growth of 5 of the 15 cultures on 4000 p.p.m. NO_3—N failed completely; 1 culture grew normally; and the remaining 9 grew somewhat irregularly. At a concentration of 2000 p.p.m. N, 3 of the 5 cultures that failed to grow at 4000 p.p.m. N grew irregularly.

A fourth series of 24 cultures has been under observation for some time, one of which persistently refuses to grow at 100 p.p.m. N and several at 1000 p.p.m. N when cultured from N-free medium. Many of the cultures grew normally at 4000 p.p.m. N when transferred from the N-free medium, and several are now growing normally at 5500 p.p.m. KNO_3—N.

DISCUSSION

Field and laboratory observations on the effect of nitrates upon the Azotobacter flora of soils tend to support the recorded observations that relatively high concentrations in a soil containing Azotobacter tend to cause a decrease in the density of the Azotobacter population. The concentration necessary to bring about this effect appears to vary rather widely with different soils, and in some soils is not particularly high.

Observations made on 65 pure cultures of Azotobacter isolated from local soils has shown conclusively that there is a marked variation in the sensitivity of different strains to nitrate nitrogen. That this sensitivity is due to the NO_3 ion and not to the cation is indicated by the fact that the same injurious effect is produced by potassium, sodium, and ammonium nitrate. That the nitrogen alone is not responsible for the phenomenon is indicated by the fact that upon the addition of nitrogen in the form of urea, the injurious effect is not evident until sufficient time has elapsed to enable the processes of nitrification to transform the urea nitrogen into nitrate nitrogen; also, by the fact that the injurious effect of ammonium nitrate is not proportional to the total but rather to its NO_3—N content.

With the toxic action of the NO_3 upon pure cultures established, it is not necessary to postulate an antagonistic action of other organisms stimulated by the nitrate to account for the reduction in Azotobacter population following an application of nitrate nitrogen to a soil. Some evidence is submitted in

connection with soils S and X in support of the view that the variation in sensitivity of the Azotobacter of different soils may be associated with the sensitivity of the particular strains inhabiting the soil in question.

Relative to the apparently much lower concentration of NO_3 that proved toxic to Azotobacter in soils, as compared with that necessary in case of pure cultures, attention should be directed to the fact that the nitrate content of soils is always recorded on a dry soil basis. Since nitrates are very soluble, such salts are probably found dissolved in the soil moisture. The actual effective concentration, therefore, as far as the Azotobacter are influenced, would vary inversely with the moisture content of the soil and in the data here presented would be from 3 to 20 times the concentration calculated upon a dry soil basis. The fact should also be kept in mind that all the pure culture studies here recorded were made upon mannite agar. Just what influence the colloidal agar-agar exerted upon the concentration of NO_3 tolerated by Azotobacter is problematic. Comparisons between the concentrations tolerated in liquid cultures and in agar cultures are under way.

Since a relatively low concentration of NO_3—N has been found toxic in soil and since the speed of the action, as observed in soil S, may be rapid, it is not impossible that the differences observed in the density of the Azotobacter population in "fertile" and "nonfertile" soils were due to differences in the NO_3—N content that existed in the pair of samples at some time. In view of the relative ease with which increased tolerance can be induced in the laboratory, however, it is difficult to see why a natural tolerance to the concentrations of NO_3 ordinarily encountered in the more fertile soils, or in soils fertilized with nitrate nitrogen, is not developed.

ON THE FORMATION OF STRUCTURE IN SOIL: II. SYNTHESIS OF AGGREGATES; ON THE BONDS UNITING CLAY WITH SAND AND CLAY WITH HUMUS

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In this paper a new theory of structure formation based on the capacity of soil colloids to form phases of the aggregate state is proposed.

Quartz sand mixed with fine particles of colloidal clay (in the state of sol or gel) assumes the structural form of clay. "Intergrowth aggregates" are formed as a result of the similarity of the crystalline periods of clay and quartz. The tenacity of these intergrowths is so great that aggregates prepared from highly hydrated Na-clay and perfectly pure quartz sand show a very remarkable resistance to water and do not differ from similar aggregates prepared from Ca-clay.

Humus is irreversibly adsorbed by clay, and this raises the tenacity of the aggregates. This increase of water-resistance, however, is not observed for every clay in the same degree; in fact, some clays are not in the least affected by humus. The binding of humus with clay is due to the selective orientation of humus particles on clay according to the type of a non-homogeneous mesomorphous system. The aggregation of colloid particles causes the deformation of ion layers around them: a compression of the ion layer takes place. As a result, these differences, conditioned by the nature of the absorbed cations, are levelled out.

Calcium influences the particles of those colloids which water affects more intensely, i.e., loosely packed particles (unstable systems) or particles irregularly distributed.

The first investigators of the problem of soil structure attached great importance to clay and humus. Schloesing (36) thought that these substances were able to cement the mineral elements in soil. The ideas of Schloesing were later developed further by Dumont (8), who suggested that the important properties of soil depend on colloid envelopes: the active colloidal material being a jelly-like or amorphous mass, enveloping solid mineral grains. This idea was afterwards supported by Hall (18), who held the opinion that since the colloidal envelope has no defined external surface it can be considered as surrounding the soil particles in a series of cells with a gradually increasing hydration, so that there is a gradual transition from the solid grain to the solution. The colloidal envelopes alter essentially the properties of mineral particles and render the soil structure cellular and reticular (19). Very

similar conclusions are suggested by Comber (5) and by Haines (17), who demonstrated by their experiments how greatly the colloidal envelope affects the properties of mineral grains and how far it aids aggregation.

There exists, however, another point of view, according to which the ability to aggregate depends on the surface water film. Manegold (26) determines the force of cohesion on the basis of the properties of a liquid ring lying between two spheres of equal size, if all excess liquid is eliminated. According to Nutting (29), the thin absorption film binds the particles into aggregates: in his words,"... the cohesive pressure may mount to a respectable figure, depending largely upon the size and shape of the grains." According to Norton (28), the thin water film plays a decisive part in the formation of the structure of clays. Clay particles attract strongly to their surface their water films, which they have in common with the adjoining particles. This phenomenon determines cohesion and lubrication simultaneously. Norton (27) thinks it possible to determine the nature of surface films by studying the mechanism of shrinkage during drying and the curves of shrinkage.

Wetting with water loosens the bonds between the particles. In this case swelling is understood as a sufficient increase in the thickness of the layer of adhesion that it reaches the value of the radius of the sphere (grain). The liquid rings between the spheres disappear during swelling and, because of this, are lost (26). According to Derjaguin (7) water lowers the friction between the crystalline lamellas. A fine-structural breaking-up of the tri-dimensional periodic structure of the crystal into a one-dimensional and a two-dimensional structure of the mesomorphous type takes place under the influence of water. For bromphenanthrene sulfonic acid this has been established by Rinne (33); for clays, by the author.

The aggregates in soil, however, do not always break up after being wet. There exist tenacious structures which are not affected by distilled water (35), and it has been found by the author that such structures are resistant to alkalis as well. The present work was undertaken for the elucidation of the phenomena of structure formation.

MATERIALS

Pure quartz sand was used for the synthesis of aggregates. The sand was ground in a ball-mill and then was freed from contamination by being washed several times with a boiling HCl solution. The acid was washed off first with tap water then with distilled water. After this, the sand was fractionated by means of elutriation. The elutriated sand was heated for several hours in a muffle-furnace, cooled in a desiccator, and stored in a glass container fitted with a ground stopper.

The clay suspensions were prepared from various rocks belonging mostly to Quaternary deposits.[1] The method of preparation of these clay suspensions,

[1] The rock samples were obtained from the collection of Professor Mirchink; two samples of clay from carbon and Jura deposits were taken from the shafts of the Moscow Underground Railway.

the data on their mineralogical composition, and a description of the humates used for these experiments have been given in part I (37).

ADSORPTION OF CLAY ON QUARTZ

Water films may stabilize the weak skeleton of sand if the excess water will flow out of the sand mound under the influence of gravity. The cohesion between the grains of sand will increase if their water film is enriched by some soluble substance, e.g., clay. Will there be a condensation of the dissolved substance on the surface of the quartz grains in the shape of an adsorbed layer, or will the layer of adsorbed molecules serve as a point of departure for chains of oriented molecules, extending into the mass of the binding substance (23, 24, 25), i.e., will a layer of adhesion determining the value of the stress necessary for disrupting the bond be formed?

Adsorption of clay by quartz has not been studied heretofore. Observations point to the possibility of the clay's condensation on the quartz in the shape of very tenacious pellicles which are not dissolved by water. It is almost impossible to obtain clay-free sand in the process of the texture analysis of soil even if the dispersion of the colloids is complete.

Anderson, et al. (1) have found, that the separation of soil into colloids and non-colloids is impossible, since colloidal material cannot be freed from fine mineral particles.

Adsorption of clay on quartz may take place on the basis of the similarity of the crystalline periods of quartz and clay. In silica the layer of the tetrahedrons of silicon oxygen has the shape of a hexagon with the length $a_0 = 5.2$ Å. Kaolinite is made up of silicon-oxygen layers, oriented in a hexagonal pattern, the distance a_0 equaling, according to the measurements of Gruner (16), 5.14 Å. A similar pattern, a hexagonal ring of three silicon oxygen tetrahedrons bound together, has been found for montmorillonite.

Adsorption was observed in almost every sample of clay taken by us. Quartz sand with a grain-size of 20–50μ was used for the experiments. The container was thoroughly cleansed with chromium mixture, and the pipette was washed with clay sol. The volume of the clay suspension reached 25–50 cc. for 5–10 gm. of sand. The time of adsorption was 1 hour. After adsorption, the suspension was filtered through a filter, which did not let the sand pass (the filtrate was examined for sand under the microscope). The value of the adsorption was determined by the final concentration of the solution. It was found that after the sand was immersed in the solution the equilibrium concentration changed somewhat. To determine whether the adsorption was actually irreversible, the powdered quartz, after the liquid was decanted, was divided into two equal parts. In one of these we determined the irreversibility of the freshly deposited clay layer; in the other, the irreversibility of this layer after a preliminary drying at 36°C. In both cases the irreversibility of adsorption was established by means of a thorough and prolonged washing of the powder with distilled water. The washing was continued until no turbidity

was apparent. Usually this washing took 3 or 4 days, the sand being left under water overnight, and the washing was terminated if on the next morning no turbidity appeared after mixing (without the use of a rod). This method was strictly adhered to in all the following experiments. After the washing, the layer of adsorbed clay was dissolved in a 10 per cent boiling HCl solution. These experiments showed that not all of the samples of freshly adsorbed clay pellicles had the property of irreversibility. The irreversibility of the clay layers after being dried, however, was very marked for all our samples.

TABLE 1

Adsorption of clay on quartz sand

CLAY	C*	a†	IRREVERSIBLE ADSORPTION	
			a_I‡	a_{II}§
	per cent			
1. Mokshany (cover clay, Riss)	2 44	1 0	0	0.42
2. Kobeliaki (fluvio-glacial, Würms)	....	. .	0 12	0 40
3. Red clay (Carboniferous)	2 24	0 42	0	0.44
4. Zhaki (loess loam, Würms)	3 68	0 76	0	1.80
5. Ostrovy (bottom moraine, Riss)	3 25	0 64	0	0 88
6. Glivin (moraine, Mindel)	2 57	0 46	0 12	0 56
7. Riazan (moraine, Riss)	2 51	1.68	‖	‖
8. Dzekan (loess loam, Würms)	4 48	0 58	0 30	0 60
9. Alatyr (cover clay, after Riss)	2 71	0 28	0	0 80
10. Toida (cover clay, after Riss)	3 10	0 85	0 56	1 50
11. Seno (fluvio-glacial clay, Würms)	4 79	0 67	0.36	....
12. Black clay (Jura)	....	....	....	0 96

* C = equilibrium concentration in gm. per 100 cc. of solution.

† a = mgm. clay absorbed per gm. of quartz sand.

‡ a_I = mgm. freshly precipitated clay per gm. of quartz sand.

§ a_{II} = mgm. clay, after preliminary drying at 36°C., per gm. of quartz sand.

‖ Not measured.

MEASUREMENT RESULTS

Table 1 shows the results of the adsorption experiments. Since several suspensions coagulated during filtration, they have not been included.

The irregularity in the properties of the crystalline surface and the existence of active centers on this surface make precise calculations regarding the monomolecular layer impossible. Moreover, not every clay of those we examined is uniform in its mineralogic composition. The order of their adsorption values, however, suggests the possibility of a monomolecular layer. A detailed consideration of this question is not necessary for the explanation of the nature of aggregation. The bond, conditioned by the force of cohesion of the molecules of the adsorbed layer, acts within a small distance, on the order of a double layer of clay crystallites. Even in a relatively fine sand, such as was taken for

the experiment 20–50μ, a bond of this character seems to be rare. Under the microscope only rare small aggregates united by thin adhesion layers are observed in sand moistened with water. The prevailing mass of sand consists of separate grains.

The data of table 1 show the increase of irreversibility under the influence of drying. This is particularly apparent in those clays the freshly precipitated layers of which proved completely reversible. Drying assists in the accumulation of clay in the irreversible state. It is possible to raise this accumulation to a certain maximum point and, with it, the force of cohesion as well. This process is due to the fact that orientation is not limited to one or two layers of molecules but extends much farther, one layer of molecules tending to orient the next, until orientation is fixed and there is no disorientation. Side by side with the formation of the adhesion layer on the crystalline surface there may be also a grouping similar to that of the mesomorphous system under the influence of the surface water film (pl. 2, fig. 1).

ON THE BOND BETWEEN CLAY AND QUARTZ SAND

The existence of a firm bond between clay and quartz may be explained in several ways. The intergrowth of clay crystallites with quartz, similar to non-homogeneous crystalline formations definitely oriented to one another, may be formed. It is possible that the displacement of quartz by kaolinite observed under natural conditions—such as the one described by Ross and Kerr (34) for a clay from Camden, Alabama—must be classed among phenomena of this kind.

Besides crystalline intergrowth the formation of non-homogeneous aggregates is possible also along the type of the mesomorphous or the paracrystalline system. The observation of Nutting, that "aggregates of fine and coarse particles, taken together, when moist assume the properties of the fine particles," refers to this type of aggregation (pl. 2, fig. 1).

The remarkable property of sand to acquire the properties of clay has been noted by Freundlich (9) also in respect of tyxotropic phenomena. Freundlich describes a case in which the tyxotropic properties of a quartz sand were determined by the presence of an argillaceous substance.

The ability of sand to assume the form of grouping belonging to clay is explained by the similarity of their forms of orientation: fine sand that has been mixed with water, when it dries gathers, under the influence of the film of surface tension, into rows resembling the structure of the smectic phase of clay [pl. 1, fig. 10, (37)]. The smectic phase of clay possesses a partially periodical structure, the periods of which coincide with the crystalline periods of quartz. Thus the condition necessary for the occurrence of selective orientation or "épitaxie" (according to Friedel, 14) arises. The tenacity of the "intergrowth" does not depend, therefore, on the nature of the absorbed bases (cations) and of the liospheres determined by them.

The tenacity of the bond between sand and clay increases with the decrease

of grain-size. It is quite impossible to separate fine sand from clay, as was long ago indicated by Schloesing (35, p. 544) and is well known from the practice of soil texture analyses. With the increase of the size of sand grains the tenacity of the bond decreases rapidly; clay can be easily washed off coarse sand, only thin adhesion layers being left on the surface of the sand grains. Experiments were therefore arranged for demonstrating the possibility of the formation of stable ("water-tight") structures from highly hydrated Na-clay.

Mixtures of clay with quartz sand (10–20μ), containing about 15 per cent of clay, were prepared. A Na-clay sol was slowly evaporated in the incubator at 35°C. until it reached the state of a very viscous jelly-like mass. Perfectly pure sand was mixed in a porcelain dish with a weighed amount of clay, and the mixture was left to dry slowly for several days at room temperature in a place protected from dust. During this drying the mass was thoroughly mixed several times with the purpose of obtaining a uniform mixture of clay with sand.

TABLE 2

Irreversibility of Na-clays mixed with sands

CLAY	CLAY CONTENT OF INITIAL MATERIAL	CLAY CONTENT OF RESIDUE AFTER WASHING WITH WATER (INSOLUBLE CLAY)	LOSS
	*mgm.**	*mgm.**	*per cent*
1 Zhaki (loesslike loam, Würms)	142 9	129 1	9 7
2. Toida (cover clay, after Riss)	151.5	58 1	61.6
3. Kobeliaki (fluvio-glacial clay, Würms)	146 2	60 6	58.6
4. Ostrovy (bottom moraine, Riss)	146 8	68 3	53.4
5. Red clay (Carboniferous)	122 6	69.7	43.1
6. Alatyr (cover clay, after Riss)	140 8	67 7	51 9

* Per gm. of mixture.

Mixtures of sand with Ca-clay were prepared in the same manner for comparison. Ca-clays were prepared by precipitating Na-clays with a normal solution of pure $CaCl_2$ and by subsequently washing the Ca-clay precipitate many times with the same $CaCl_2$ solution. The Ca-clay precipitates were thoroughly dialyzed so that they would contain no water-soluble salts. The clear supernatant liquid after sedimentation was siphoned off.

When the weight of the mixtures of clay and sand reached a constant value, experiments were set up for the determination of the amount of firmly bound clay, i.e., clay insoluble in distilled water. Two grams of each of the mixtures of sand with Na- and Ca-clays, calculated as dry weight, was thoroughly washed with distilled water according to the technique just described until clay ceased completely to dissolve. Clay, adhering tenaciously to the grains of sand, was determined after it was dissolved with hydrochloric acid. The sediment washed with water was treated with acid on a water bath until the

clay was completely dissolved, after which the sediment was washed on a filter by a hot solution of acid. If not all the clay had been dissolved, as could be seen from the coloring, the sediment was again transferred, together with the filter, into a porcelain dish and the clay was dissolved in aqua regia. Usually the washed sediment after being heated in a muffle furnace was perfectly white.

Table 2 shows the results of the determinations of the insolubility of Na-clays mixed with sand.

A remarkable result of these experiments on synthetizing aggregates is that we were able to obtain a tenacious water-tight structure from highly hydrated Na-clays. This phenomenon is in contradiction to, and cannot be explained by, the existing conceptions regarding the nature of soil colloids and the direct participation of adsorbed Ca in the process of structure formation. It should be noted that the possibility of the formation of an isoelectric precipitate in this case is excluded when sand is mixed with clay. One might consider hysteresis in this connection, but our observations on the swelling of Na-clay dried at 100°C. show that in some cases (Toida, Alatyr) the clay is dissolved. Moreover Na-clay sols dried at 100°C. are, without exception, soluble in water.

THE ACTION OF ALKALI ON TENACIOUS AGGREGATES OF SAND AND Na-CLAY

Alkalis are considered to increase the dispersion of clay, but the general conception among soil scientists concerning the influence of OH ions is not applicable to this case (6), since we are dealing here with Na-clays containing highly hydrated Na ions. According to Wiegner (40) the ζ-potential of such a clay, as well as its degree of dissociation, must be high. The addition to the Na-clay of an electrolyte (NaOH) possessing a cation in common with the clay particle, must result in a shrinking of the double layer, i.e., in a decrease of the distance between the particles, and in flocculation (20). This, however, does not occur; on the contrary, an increase of solubility is observed. This increase of solubility can be most probably explained by the decrease of external friction between the lamellar particles of clay under the influence of the NaOH solution, a phenomenon established by Derjaguin for mica (7).

The arrangement of the experiments did not differ from the foregoing. Tenacious aggregates remaining after washing with distilled water were treated with a 0.05 N solution of NaOH. Washing with the same solution was continued until turbidity was no longer apparent above the sediment. After the alkali treatment, the sediment was washed with distilled water. All the clays generally produced some turbidity after the addition of distilled water. In some of them this turbidity was insignificant and disappeared quickly after washing. The shrinkage of the ion envelope under the influence of NaOH and the subsequent lessening of this shrinkage under the influence of distilled water seem actually to take place. The results of this experiment are summarized in table 3.

The experiments show that even treatment with 0.05N NaOH does not

destroy the aggregates entirely; on the contrary, the action of alkali on some clays is hardly different from that of pure water.

THE SOLUBILITY OF CA-CLAYS

From the viewpoint of the mechanism of coagulation and of the theory of ion exchange, the density of the ion envelope in Ca-clays occupies an intermediate position between highly hydrated monovalent ions and non-hydrated mono- and polyvalent ions. The value of the ζ-potential in Ca-clay is sufficiently high for the clay to be soluble. According to Baver (3) Ca-clay is even more hydrated than Li-clay. Our observations corroborate this assumption. Only the kinetics of this process are different: Ca-clay dissolves at a slower rate. Whereas in Na-clay the separation of the soluble from the insoluble fraction requires 3 to 5 days, in Ca-clay a slight turbidity is observed even after 15 days of washing. The results of our determinations are shown in table 4. Adsorbed Ca does not prevent the solution of clay. The percentage of Ca-clay lost during washing with water is almost the same as that of Na-clay. In some cases the solubility of Ca-clay even exceeds that of Na-clay.

TABLE 3

The influence of 0.05 N NaOH on the solubility of clay

CLAY	CLAY CONTENT OF THE RESIDUE AFTER WASHING WITH WATER (INSOLUBLE CLAY)	CLAY CONTENT IN THE RESIDUE AFTER TREATMENT WITH 0.05 N NaOH	LOSS
	*mgm.**	*mgm.**	*per cent*
1. Zhaki (loesslike loam, Würms)	129.1	117.6	8 9
2. Toida (cover clay, after Riss)	58 1	38 9	33 0
3. Kobeliaki (fluvio-glacial clay, Würms)	60 6	47 2	22 1
4. Ostrovy (bottom moraine, Riss)	68 3	†	†
5. Red clay (Carboniferous)	69 7	†	†
6. Alatyr (cover clay, after Riss)	67.7	62.3	8.1

* Per gm.

† Not measured.

TABLE 4

Comparison of the insolubility of Ca- and Na- clays

CLAY	Ca-CLAY CONTENT OF INITIAL MATERIAL	Ca-CLAY CONTENT OF RESIDUE AFTER WASHING WITH WATER (INSOLUBLE Ca-CLAY)	LOSS OF Ca-CLAY	INSOLUBLE Na-CLAY	LOSS OF Na-CLAY
	*mgm.**	*mgm.**	*per cent*	*mgm.**	*per cent*
1. Kobeliaki (fluvio-glacial clay)	111.6	61 8	44 6	60 6	58 6
2. Ostrovy (bottom moraine)	126.1	57 9	54 1	68 3	53 4
3. Red clay (Carboniferous)	152.4	109.4	28 2	69.7	43.1
4. Mokshany (cover clay, Riss)	99.6	83 2	16.5	....	....

* Per gm.

Water acts more strongly on clay particles that are loosely packed and on particles irregularly distributed. They are protected from being washed off, not by absorbed calcium, but by Ca in the form of bicarbonate ions, as was long ago pointed out by Schloesing (35, p. 593): "La présence du calcaire n'aura pas préservé l'argile de l'entrainement, si le lavage a eu lieu avant que la végétation, source des débris qui fournissent l'acide carbonique et par suite le bicarbonate de chaux, se soit emparée du terrain."

ADSORPTION OF HUMATE ON QUARTZ SAND

The adsorption of humate on quartz sand, like that of clays, has not heretofore been studied. There seem to be no grounds for expecting that sand will adsorb humus, since quartz is an acid. On the other hand, experiments with the adsorption of organic substances on quartz have been described by many authors. The adsorption of proteins has been studied thoroughly (10). A marked alteration of the wetting ability occurs during the adsorption of hydrophobe substances—a fact of special interest (11). Recently the question has been studied in great detail by Lindau and Rhodius (22). They found that the adsorption of proteins on quartz follows the laws of Langmuir.

For the experiments with the adsorption of humates, the same quartz sand was used as for the experiments with clay. The volume of peat humate was 25–50 cc. for 5–10 gm. of sand. The time of adsorption was 1 hour. The experiments were arranged as previously described. Adsorption was determined by analyzing the equilibrium concentration of the humate. These experiments, repeated several times, showed no alteration in the equilibrium concentration. Following this experiment, one on the influence of drying was set up. Quartz powder, moistened with humate (concentration 1.92 gm. per liter) followed by elimination of excess liquid, so that only the adhesion rings remained, was dried at 35°C. The dried powder was washed thoroughly with water for several days. As there is no turbidity with humate, the washing was continued until the color of the sand remained unchanged. It was apparent, after this washing, that considerable quantities of humate adhere to the sand—1.58 mgm. per gram of sand. To determine the maximum amount of humate adhering to sand, a mixture of sand with peat humate, containing 12.08 per cent of humus, was prepared. The mixture was dried in the open at 35°C. then thoroughly washed with water. The amount of insoluble humus firmly adhering to sand was 5.8 mgm. per gram of the mixture.

Since the peat humate aggregates considerably less readily than do clays, one would expect that the mixture of sand and Ca-humate would prove more resistant to water than the mixture of sand and Na-humate. Indeed, the experiment showed that Ca-humate adheres to sand in greater amounts than does Na-humate. A mixture containing 12.5 per cent of Ca-humate was prepared, dried in the open, and thoroughly washed with distilled water until it ceased to lose humate. The amount of humus firmly adhering to the sand equaled 28.9 mgm. per gram of sand, consequently about 77 per cent of the

humus had been dissolved. Hence the organic substance of soil can be readily separated from sand, as has been indicated by Schloesing (35).

The situation is quite different in regard to the relation between humus and clay. As Schloesing says, "... l'argile est un ciment pour les éléments sableux de la terre, elle les relie et leur donne de la consistance" (35, p. 521), and further, "... le ciment minéral est, en quelque sorte, cimenté par le ciment organique" (35, p. 539).

ON THE BOND BETWEEN HUMUS AND CLAY

A certain amount of organic substance is most firmly bound with clay. A remarkable property acquired by clay under the influence of humus is the decrease of its solubility. Schloesing (35), on the basis of his observations, thought that "the colloids of humus take up clay, as it were, in a network thus robbing it of the capability to dissolve under the influence of water.... Clay carries away humate, while dissolved humate keeps clay back." Schloesing considered, therefore, that humus and clay form some "undefined compounds."

Recently the careful experiments of Tiulin (38) on the isolation of humates from organic-mineral gels showed that even with the aid of 0.05 *N* NaOH it is impossible to separate humus from clay completely.

Experiments performed in the course of the present work show that clay without previous drying is able to bind humate irreversibly. Thus there is a firm adsorptional bond between clay and humus. However, the accumulation of humus may be considerable, greatly exceeding the quantity necessary for the formation of the adsorption layer. A phenomenon similar to that in the case of clay-quartz sand mixtures takes place here. Mutual orientation, or the ability to form non-homogeneous aggregates, may be explained by the identity of the smectic phases of clay and humus, i.e., by their both being capable of mesomorphous aggregation. Rinne (32) is of the opinion, that "paracrystalline adsorption"[2] Phenomena extend not only to the group of regular intergrowths [i.e. those following the laws of Macles (13)], but to that of phenomena representing various kinds of stratification, such as the distribution of water and other substances in the zeolites.

For these experiments we prepared mixtures of humus with clay. In addition, we investigated the "organic-mineral" sediments after the separation of humates with a solution of KCl. The samples, according to the data of Tiulin, from whom the samples were obtained, contain much humus bound with clay. It is possible, however, to separate some humus by treatment with 0.05 *N* NaOH. As a result, clay is obtained containing almost completely insoluble humus. Such clays were also tested. In some cases a remarkable picture of dendritic-like branchings, indicating a rapid growth of crystals, may be observed under the microscope with crossed Nicol prisms. The structure obtained reminds one only remotely of the structure of the original

[2] Rinne uses the term "paracrystalline" instead of "mesomorphous" to emphasize the natural close relationship between the conceptions of "crystal" and of "paracrystal."

material (pl. 2, figs. 2, 3, 4). It is possible to see the dendritic branches radiating from a common center and to produce, in the same way, spherulites in parallel light with crossed Nicol prisms, the dark cross of the single-axis crystal keeping its position unchanged at the rotation of the stage of the microscope. The cause of this is the radial disposition of the filaments in respect to the center, readily visible in figure 4, plate 2. In the lucid quadrants of the spherulite cross in a gypsum plate of the first order it is possible to establish the negative optical character of the formation. In some parts of the preparation the transformation of the negative sign of double refraction into the positive one may be observed. According to Vorländer (39) this alteration of the optical character is related to the different arrangement of the molecules in the elementary cell. In gypsum of the first order a remarkably beautiful coloring of the different sections of the preparation and the mutually opposed transition of the colors at the rotation of the microscope stage, indicating twinning, are observed.

The structures represented in figure 5 (prevalence of humus) and figure 16 (prevalence of clay), plate 2, are characteristic of most clay-humus mixtures. As a rule, humus decreases the double refraction of clay. No sign change is observed here. Spherulites, not observed in clay, appear in the mixtures and show, in covergent light, the dark cross of one-axis crystals. The characteristic "oily strip" structure is transformed into a fluid one (pl. 2, fig. 6). The decrease of double refraction is observed also in clay samples which were allowed to swell in humate solutions. "Grade drops" ("gouttes à gradins" of Grandjean, 12) are visible in natural light in the "hollows" between the noticeably twinned smectic lamellas (pl. 2, fig. 7).

It is hardly possible to establish the distinctive features characterizing mixtures with a greater or lesser humus content. "Stratification" of humus on, or its "intergrowth" with, clay proceeds to a certain limit, and excess humate does not affect the structure of the mixture. This is suggested by a comparison of the structures observed in the sediments isolated from soil and representing an "inseparable mixture" of humus and clay with those observed in artificially prepared mixtures. Figure 9, plate 2, shows the structure in natural light of such an "inseparable sediment" of clay with humus, isolated from soil; figure 10 shows the same preparation in polarized light; and figure 11, the structure of an artificial mixture of clay with humus. Figure 11, plate 2, shows excess organic substance being drawn out to the periphery by a stronger tension than that in the clay surface (31). Only the most external rows of micellae form the smectic phase. Close to this oriented layer lies a zone showing no double refraction (amorphous organic substance). Inside of the amorphous layer lies the smectic substance with a dendrite structure, which represents the "inseparable" combination of clay and humus. The amorphous organic substance is readily dissolved as soon as the layer of oriented particles surrounding it is destroyed.

The sediments isolated from soil and representing an inseparable combination of humus and clay have a different structure from that of mixtures in that

here there is no amorphous readily dissolving layer of organic substance at the periphery. Here the smectic substance of a dendrite structure is surrounded by a zone of intense double refraction (pl. 2, fig. 10).

DECREASE OF THE SOLUBILITY OF CLAY UNDER THE INFLUENCE OF HUMUS

Many authors have expressed their opinion on the protective rôle of humus (30), but no adequate theory of this phenomenon has been supplied. The protective influence of humus was considered as hindering the coagulation of mineral suspensions. Buzagh (4) has discovered the stabilizing effect of humus on kaolin suspensions. The explanation of these phenomena is possibly connected with the alteration of the moisture capacity under the influence of humate adsorption. The protective effect of proteins on bentonite suspensions in the experiments of Ghosh (15) may be explained by a transformation of the hydrophilic surface of bentonite into a hydrophobic one. Bartell and Walton

TABLE 5

Accumulation of clay by repeated condensation

CLAY	CLAY CONTENT IN INITIAL SAMPLE a	CLAY CONTENT AFTER WASHING WITH WATER a_1	LOSS	
	*mgm.**	*mgm* *	*mgm.**	*per cent*
1. Dzekan	20 64	3 63	17 01	82 4
2. Red clay	8 5	0 86	7 64	90 0
3. Toida	21 41	4 58	16 83	78 6
4. Kobeliaki	23 73	3 00	20 73	87 4
5. Ostrovy	12 37	..	.	. .
6. Mokshany	13 49	0 72	12 77	94 6

* Per gm.

(2) point to the possibility of an arbitrary alteration of the wetting of the same surface., There are some observations the extension of which to humus seems justifiable in a certain measure. Thus, in the experiments of Kruyt (21) a tannin film transforms many hydrophilic colloids into hydrophobic ones. Kruyt affirms, that the molecules of tannin may be regarded as polar ones, and they orient themselves in such a manner that their hydrophobic ends are turned in the direction of water. According to the data of Lindau and Rhodius (22) the moisture capacity of quartz powder decreases according to the amount of albumin covering the powder, and when the albumin reaches 50 per cent the powder rapidly passes from the hydrophilic into the hydrophobic condition.

The purpose of our experiments was to establish the decrease of the solubility of clay pellicles on quartz sand under the influence of humus. The most unfavorable conditions in respect of the solubility of clay envelopes on sand were chosen for these experiments: thick, readily soluble envelopes were prepared. As in the preceding experiments, Na-clay was used. Quartz sand

moistened with the clay suspension was dried at 35°C. after the elimination of excess liquid, moistened with a clay sol, and again dried. This manipulation was repeated several times, and, indeed, as the determinations of the amount of clay have shown (table 5) the envelopes obtained were much thicker than those of the first experiment (table 1). It was found that simultaneously the amount of clay firmly bound to the sand (insoluble clay) had also increased. As may be seen from these figures the sand contained readily peptizable clay. The experiments for the determination of the influence of humus on the decrease of the solubility of clay were made with this sand.

The volume of the humate solution equaled 60 cc. for 6 gm. of the sample, calculated on the basis of absolutely dry weight. The time of adsorption was 1 hour, during which the sand was thoroughly mixed with the humate solution. After this, the liquid was filtered through a filter which retained all the sand. The concentration of the equilibrium solution (C), the adsorption of humate,

TABLE 6

Reciprocal relation of the adsorption of humus and clay

CLAY	C	HUMATE ADSORBED	AMOUNT OF CLAY DISSOLVED IN HUMATE	REMAINING CLAY, ABLE TO PEPTIZE IN WATER
	per cent	*mgm* *	*mgm.**	*mgm* *
1. Dzekan	0 192	0	2 08	14.93
2. Red clay	0 186	0 22	2 08	5 56
3. Toida	0 190	0 08	0 56	16 27
4. Kobeliaki	0 192	0	2 08	18 65
5. Ostrovy	0 188	0 15	3 12	.....
6. Mokshany	0 185	0.29	0 32	12 45

* Per gm.

and the amount of clay which had been dissolved in the humate were then determined (table 6).

The adsorption of humate by clay, generally only an insignificant value, was not observed at all in some of the clays. Adsorptional layers hardly reach the values necessary for even a monomolecular layer. Accordingly the determination of the decrease of the solubility of clay under the influence of freshly deposited humate was not undertaken. We decided to repeat this experiment using a thicker layer of clay. To form a more compact layer of humate on the clay envelopes, the sand, remaining after the adsorption experiment, was freed from excess humate solution by suction with a water-pump and then dried at 35°C. The dried sand was again moistened with humate and again dried, i.e., the same manipulations as with clay were repeated with humate. Thus the clay layer able to peptize in water was "protected" by a thicker layer of humus. The decrease of solubility is easy to determine if one compares the amount of soluble clay (table 6) with the amount remaining on

quartz after washing with water (table 7). The sand, covered with clay and humus, was washed with water according to the technique previously described. The dissolved humate was determined by difference: the initial material and after it had been washed with water. The dissolved clay was determined by the amount remaining after washing. The remaining insoluble clay was determined, as before, by dissolving in HCl solution. A necessary correction was made in the value obtained to allow for the humus contained in the insoluble clay.

The results of these experiments show that the solubility of clay is decreased under the influence of insignificant amounts of humus—and that drying increases the irreversibility of the humate. A possible explanation of this is the formation of an adhesion layer and the orientation of the molecules at the surface of quartz sand mixed with clay.

TABLE 7

The influence of humus on the decrease of the solubility of clay

CLAY	HUMUS CONTENT OF INITIAL MATERIAL	HUMUS CONTENT AFTER WASHING WITH WATER	CLAY CONTENT AFTER WASHING WITH WATER (IRREVERSIBLE CLAY)*	LOSS		DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm.**	*mgm.*	*per cent*	*per cent*
1. Dzekan	2.9	2 55	5.18	10 75	67 4	15.0
2. Red clay	2 0	1 74	2 15	3 41	61 3	28 7
3. Toida	2 04	1 63	10 38	5 89	36 2	12 4
4. Kobeliaki	3 34	1 0	11 13	7 52	40.3	47.1
5. Ostrovy	4 20	0 65	11 7	.	....	....
6. Mokshany	4.40	2 0	1 53	10 92	87 9	6 7

* Per gm.

DECREASE OF CLAY SOLUBILITY UNDER THE INFLUENCE OF FRESHLY DEPOSITED LAYERS OF HUMATE

Samples of quartz sand-Na-clay mixtures which had been used for the study of the tenacity of the bond between Na-clay and quartz sand (table 2) were taken for these experiments. The phenomenon of the adsorption of humate should be still more evident in this case because of the greater content of clay. The volume of humate used was 60 cc. for 1 gm. (absolute dry weight). The time of adsorption was 1 hour, during which the material was mixed several times. After this, the liquid was filtered, and the concentration of the equilibrium solution was determined. The residue was washed with water immediately after filtration until no turbidity appeared in the filtrate. After the washing was completed, the sediment was dried and the content of undissolved (irreversible) humus and that of undissolved (irreversible) clay were determined. The results are summarized in table 8.

The decrease of the solubility of clay is related to the adsorption of humus but does not show an equal correlation with it in all the tested samples of clay.

Apparently the mineralogical composition and the influence of contaminations play a certain part in this matter. In some cases the adsorption of humate produces no change in the solubility of clay (clay "Zhaki") or only slight changes.

INFLUENCE OF DRYING

The experiment was modified in comparison with the preceding experiment. The samples used were the same, but the saturation with humate was brought about by letting the material swell in humate solution. The samples were

TABLE 8

Adsorption of humus on clay

CLAY	HUMUS ADSORBED	IRREVERSIBLE HUMUS ADSORBED	CLAY CONTENT AFTER WASHING WITH WATER (IRREVERSIBLE CLAY)*	LOSS	DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm.*	*per cent*	*per cent*
1. Zhaki	1 83	1 64	117.6	18.0	0
2. Toida	. . ..	1.64	96.0	36 6	25.0
3. Kobeliaki	1 59	1 50	68 7	53 6	5 6
4. Ostrovy	1 34	0 98	80 0	45 5	7.9
5. Red clay	1 27	0 92	....	..	....
6. Alatyr	1.16	0 91	103.9	26 2	25 7

* Per gm. of mixture.

TABLE 9

Influence of humus drying on the solubility of Na-clay, mixed with sand

CLAY	HUMUS CONTENT IN INITIAL MATERIAL	IRREVERSIBLE HUMUS	IRREVERSIBLE CLAY	LOSS OF CLAY	DECREASE OF SOLUBILITY
	*mgm.**	*mgm.**	*mgm* *	*per cent*	*per cent*
1. Zhaki	12 8	6.1	118 4	17 1	0
2. Toida	15.1	7.4	118 8	21 5	36 6
3. Ostrovy	9.5	9 3	107 4	27.0	41 3

* Per gm.

placed on glass slides covered with filtering paper, the ends of which were immersed in a humate solution. As the evaporation from the surface of the samples was not eliminated (the container with the swelling samples was placed in a large crystallizer covered with filter paper to protect it from dust) the saturation with humate constantly increased. After 15 days the supply of humate was removed and the samples were left to dry slowly at room temperature. All the samples during the swelling showed a fine structure with horizontal fissures forming a number of parallel lamellas. In drying, these samples acquired the ability to fall into small clumps, reminding one by their

shape of the natural structure of soil, unlike the viscous consistence of the samples of sand—Na-clay mixtures. Moreover, these clumps proved tenacious and water-tight. Otherwise, the study of these samples differed in no way from the preceding. The samples were treated with water to eliminate all soluble matter (i.e., clay and humus). After this washing, the remaining humus and clay were determined, and their amounts were compared with those in the initial material (table 9).

Drying increases the accumulation of humus in an irreversible condition. Simultaneously, the solubility of clay is further decreased. Again the fact stands out that on some clays, humus has no effect.

DISCUSSION OF RESULTS

The drawing together of the colloidal particles, which occurs during the formation of the phases of the aggregate state, leads to a deformation of the ion envelope around these colloidal particles. This deformation is expressed in a compression of the ion layer or in a decrease of the distance of the double layer, and must affect all the properties, depending on the dimension of the double electric layer. The density increase of the ion layer decreases the value of the ζ-potential, decreases the degree of dissociation, and consequently must decrease the solubility and exchange capacity.

In our experiments the decrease of the solubility of Na-clay mixed with sand and humus depends on the compactness of the particles. A homogeneous arrangement of the particles corresponds to the most compact condition and, therefore, is the most stable one. An accumulation of irregularly arranged particles and a loose (non-homogeneously orientated) packing represent an unstable system, the properties of which depend on the dimensions of the ion layer. The conceptions relating to the theory of the colloidal micella as a surface with a double electric layer are essential for the explanation of the properties of this system. The properties of such an unstable system depend in a high measure on the nature of the absorbed bases. The shrinking of the ion envelope at the grouping of the particles levels out the difference due to the kind of absorbed cation, and explains the equal solubility of the aggregates prepared from Na-clay and from Ca-clay. Hence the notions existing in soil science in regard to the behavior of soil colloids proved to be only partly correct, since they do not take into account the ability of soil colloids to form phases of the aggregate state. The instability of Na-clay suspensions is also easily explained by the deformation of the ion envelope at the association of particles into groups. According to Jenny, the curve representing the ratio between the ζ-potential and flocculation is in the form of a parabola, showing that the stability of a clay suspension increases several times at even a slight rise of the ζ-potential. The suspension proves stable only within a narrow interval of the ζ-potential, ranging between 60 and 40 m.v. At ζ-potential = 40 m.v., clay coagulates in the absence of electrolytes. This tendency of clay suspensions to flocculate is explained by the ability of clays to form phases of the

aggregate condition. Because of this, clay coagulates more readily than humus, of which the capability to aggregate is much weaker.

The depression of the ion layer in the aggregated particles results also in a decrease of the exchange capacity. This decrease is always observed during the determination of the absorbing capacity of stable aggregates. According to the data of Tiulin the absorbing capacity of the so-called "isoelectric group of gels" is always lower than that of the first fraction of "electronegative" gels. In the same measure, the decrease of the absorbing capacity is characteristic of the so-called "passive" slime of Sokolovsky.

The cohesion between the individual parts of a non-homogeneous aggregate consisting of colloidal particles and of particles of a greater size may be explained by the orientation of the molecules of the layer of adsorption only in the limited case of the closest contact between the coarse mineral elements. In most cases the orientation of the molecules of the layer of the cementing substances extends to a certain depth, there to become disorganized and to be replaced by the smectic elements of the grouping. The increase of the stability of the bond and the decrease of the solubility of the clays cannot be explained only by the influence of the adsorbed layer of humus and by the decrease of moisture capacity due to a definite orientation, as is recognized, for instance, in regard to the molecules of fatty acids. In soil we are dealing with a large accumulation of humus in an irreversible state and with the prevalence of the variable forms of the mesomorphous grouping.

It is striking, indeed, that there can be highly stable (i.e., insoluble in water) non-homogeneous aggregates composed of parts which are separately readily soluble in water. The sol of Na-clay after it has been dried at 100°C. readily dissolves again in water, but, mixed with pure quartz sand, it becomes insoluble and acquires the character of a very tenacious cement. A similar behavior is manifested by humus-clay aggregates obtained from a Na-clay and Na-humate material, which readily peptizes under the influence of water. These aggregates acquire a remarkable resistance not only to water, but also to alkalis.

SUMMARY

The former conception that the particles forming the soil structural aggregate are held together by the tension of the water film is supplemented by the finding that the surface film creates a definite orientation of the particles in relation to one another.

The tenacity of the bond between the separate parts of the soil structural aggregate cannot be explained by the presence of absorbed Ca. The explanation of the tenacity of this bond lies in the stability of the group arrangement of the particles. The most stable arrangement of particles is the homogeneous one.

The formation of non-homogeneous aggregates in soil—sand-clay and clay-humus—may be explained by the same laws which have been found to exist for mineral intergrowths.

The impossibility of completely separating soil into its elementary parts is corroborated. In connection with the theory developed, it becomes necessary to revise the theory of soil texture. The subdivision of soil into stable and unstable groupings of particles appears to be more expedient.

The influence of external conditions (vegetation, microorganisms, tillage, pressure, freezing, etc.) on the formation of structure can be explained by the swarm theory.

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PLATE 2

MICROPHOTOGRAPHES OF CLAY-SAND AND HUMUS-CLAY MIXTURES

FIG. 1. Clay-sand mixture in polarized light. Magnification ×3.

FIG. 2. Humus-clay mixture. Dendrites. Polarized light. Magnification ×40.

FIG. 3. Another position of the preparation shown in figure 2. Magnification ×40.

FIG. 4. Another position of the preparation shown in figure 2. Spherulite of a mono-axis crystal. Polarized light. Magnification ×40.

FIG. 5. Humus-clay mixture. Non-homogeneous structure. Oily stripes and spherulites of a mono-axis crystal. Polarized light. Magnification ×27.

FIG. 6. Humus-clay mixture. Fluidal structure. Polarized light. Magnification ×27.

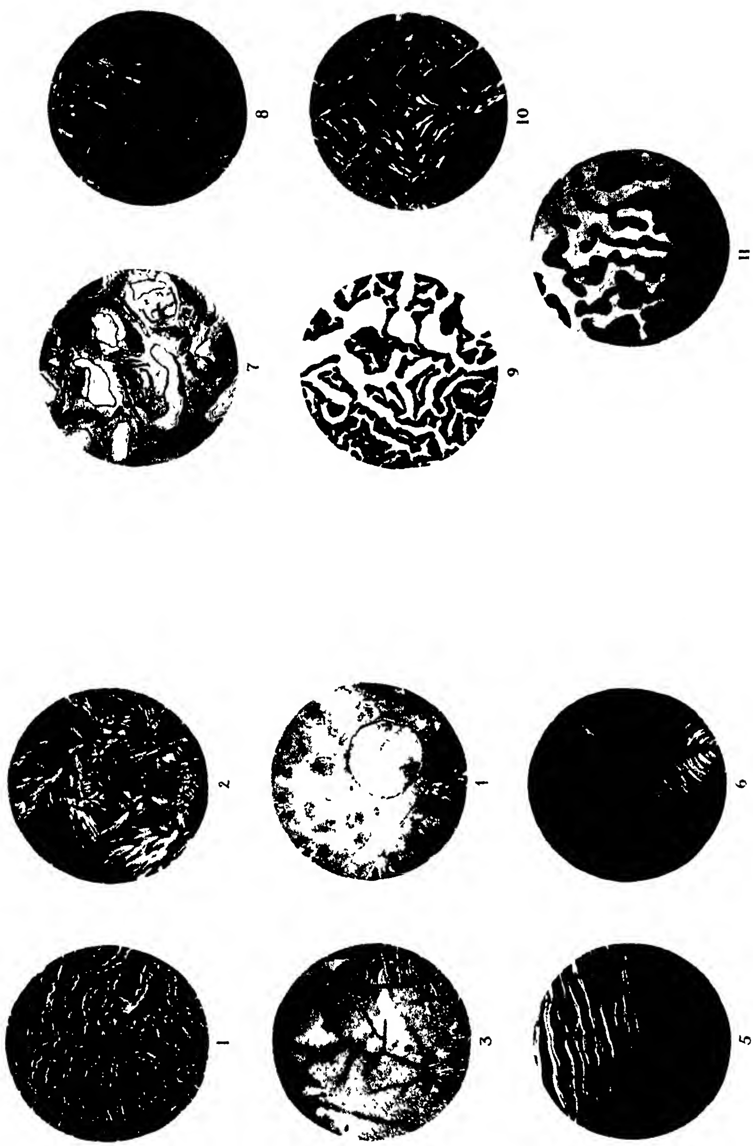
FIG. 7. Clay "Toida." Swelling in humus. "Grade drops" of Grandjean. Natural light. Magnification ×27.

FIG. 8. The preparation shown in figure 7 in polarized light. Magnification ×27.

FIG. 9. Structure of "inseparable" humus-clay precipitate. Natural light. Magnification ×27.

FIG. 10. The preparation shown in figure 9 in polarized light. Magnification ×27.

FIG. 11. Artificial mixture of humus with clay. Natural light. Magnification ×27.



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